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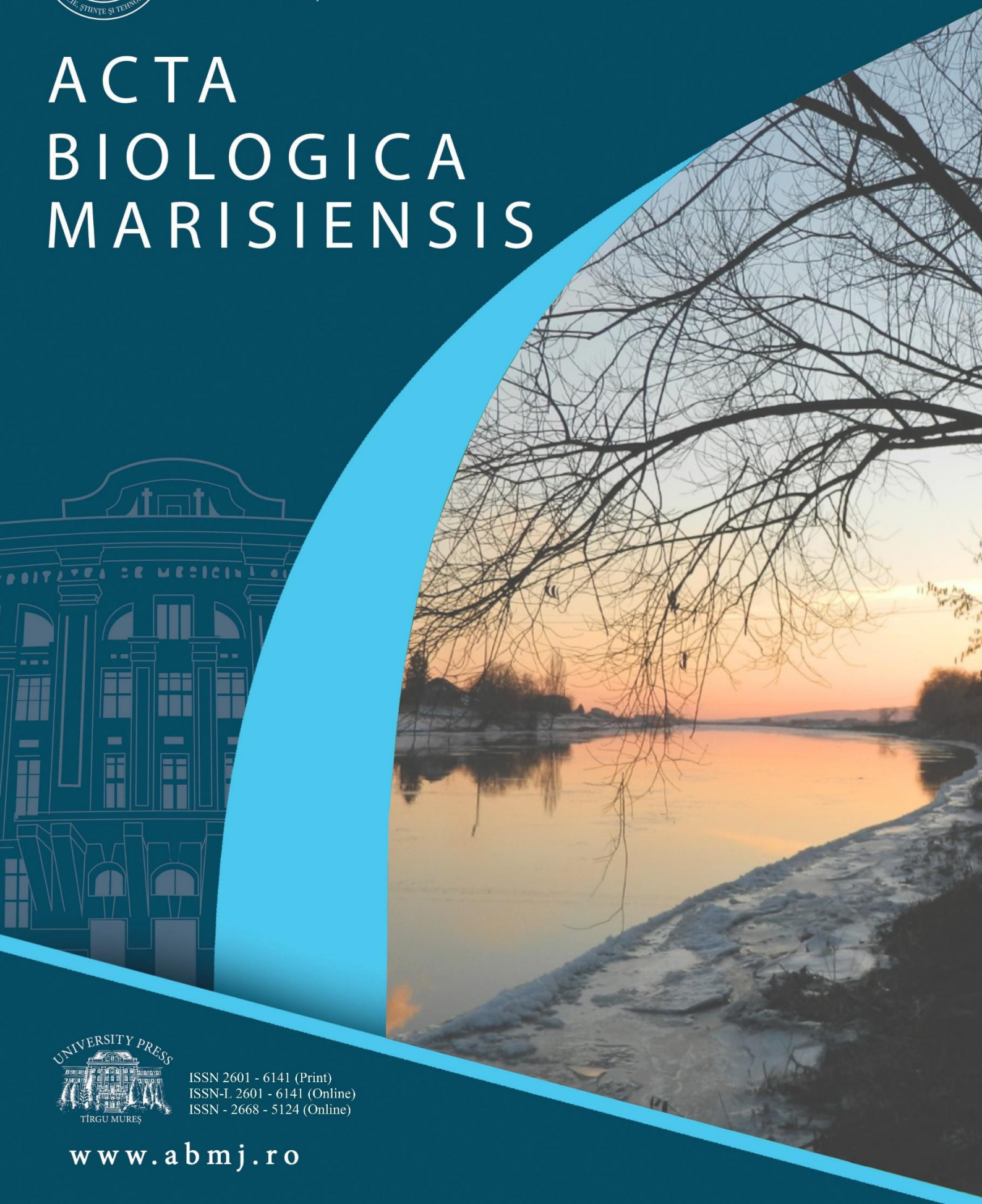

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NEW INSIGHTS INTO THE PHARMACOLOGICAL MANAGEMENT OF RHEUMATOID ARTHRITIS

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Abstract: Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease characterized by synovial inflammation and progressive joint destruction which significantly impairs patients' quality of life and generates substantial socioeconomic costs. Conventional disease-modifying antirheumatic drugs (DMARDs) such as methotrexate are the cornerstone of RA therapy, particularly for early or mild forms of disease. The newer, biologic DMARDs are a more advanced therapeutic option when conventional DMARDs are not sufficiently effective, or they cause important adverse effects. Targeted synthetic DMARDs are also a recently authorized class of drugs which have the advantage of oral administration. The aim of this review is to provide an overview of rheumatoid arthritis' pharmacological management, focusing on the most important properties of conventional, biologic and targeted synthetic DMARDs and presenting also the current international treatment guidelines. Natural compounds which could be used as adjuvants in the therapy of RA are also detailed.

Keywords: rheumatoid arthritis, conventional DMARDs, biologic DMARDs, targeted synthetic DMARDs, natural compounds

1. Introduction

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease characterized by synovial inflammation, progressive joint destruction, and various extra-articular manifestations, such as rheumatoid nodules, renal and cardiovascular impairment, pleurisy or anemia (Smolen et al., 2016; Das and Padhan, 2017). Rheumatoid arthritis can affect approximately 1% of the global population, significantly impairing patients' quality of life and generating substantial socioeconomic costs due to chronic disability and comorbidities (Gibofsky, 2012). Over 50% of patients cease professional activities within 5 years of diagnosis, and 10% develop permanent joint deformities within 2 years if untreated (Gibofsky, 2012; Picerno et al., 2015).

The advent of new types of disease-modifying antirheumatic drugs (DMARDs) has revolutionized RA management enabling

remission or lowering disease activity in many patients.

Early diagnosis and effective treatments are critical to prevent irreversible joint damage and improve long-term outcomes (Picerno et al., 2015; Smolen et al., 2016). Nevertheless, the high cost of modern DMARD drugs may limit their availability in developing countries, highlighting the importance of the use of all available therapeutical resources including phytochemical remedies used in traditional medicine which could be significant in certain geographical regions.

This review is aimed at providing an overview of the current understanding of RA, with an extensive emphasis on conventional, biologic and targeted synthetic DMARDs, but also on possible phytotherapeutical alternatives.

2. Materials and Methods

This review used scientific articles written only in English, published between 2015-2025, identified in PubMed, Scopus, Elsevier and Web of Science databases. The search used keywords like "rheumatoid arthritis", "rheumatoid arthritis AND conventional disease-modifying antirheumatic drugs", "rheumatoid arthritis AND biologic disease-modifying antirheumatic drugs", "rheumatoid arthritis AND phytotherapy".

3. Rheumatoid arthritis: epidemiology and pathogenetic mechanisms

Rheumatoid arthritis (RA) has a global prevalence of 0.5-1%, with a male-to-female ratio of 1:2. The prevalence of RA shows a geographical variability with higher values in Western and Northern Europe and North America and lower values in East Asia and Africa, due to genetic and environmental factors (Cross et al., 2014; Smolen et al., 2016). Over the last three decades there has been a significant increase of RA-related incidence rate and RA-related disability-adjusted life years (DALYs), highlighting the significant burden of the disease, which is now considered a significant global health problem (Zhang et al., 2025). Additional comorbidities may complicate the evolution of the disease and also, mortality rates are still higher in RA patients compared with general population, although they have decreased in the last decade (Abhishek et al., 2018).

RA is a complex disease characterized by the interaction of genetic and environmental risk factors which alter the immune tolerance of affected patients (O'Neill et al., 2024). Genetic pre-disposition, particularly the existence of certain Human Leukocyte Antigen alleles (HLA-DRB1), is a major risk factor, present in over 80% of RA patients, being associated with severe disease and bone erosions (Picerno et al., 2015; O'Neill et al., 2024). Environmental factors, including smoking, alcohol consumption, inflammatory diet and infections also contribute to RA's development (Smolen et al., 2016; O'Neill et al., 2024).

The abnormal immune response from RA usually starts in tissues and organs distantly situated from the synovial joints, like the lungs, gums or gastrointestinal tract, where certain proteins are modified by citrullination, carbamylation and acetylation, which later will become the target of autoantibodies (McInes and Schett, 2017). According to multiple evidence, several years before the development of clinical symptoms, patients with RA show increased blood levels of auto-antibodies like rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA) but also the lesser discussed anti-carbamylated protein (anti-CarP), and anti-malondialdehyde-acetaldehyde (anti-MAA) (O'Neill et al., 2024). Generally, around 80% of all patients with RA are considered "seropositive", presenting

detectable levels of autoantibodies like RF and ACPA (Wu et al., 2021).

The pathogenesis of rheumatoid arthritis involves a complex immune dysregulation in which aberrantly activated immune cells play a central role, diffusely infiltrating the synovium. In RA, T lymphocytes (CD4+ Th1, Th17) and B lymphocytes drive inflammation, producing pro-inflammatory cytokines (tumor necrosis factor-TNF-α and interleukins IL-6, IL-17) that promote synovial hyperplasia, pannus formation, and osteoclast activation, leading to joint and bone destruction. Additionally, the proinflammatory cytokines can induce acute-phase responses (e.g., elevated C-reactive protein) and may cause systemic complications like anemia (via hepcidin induction), and osteoporosis (via osteoclast activation), contributing to the significant comorbidities of RA (McIness and Schett, 2017; Alivernini et al., 2022).

4. Pharmacological management of rheumatoid arthritis

The drugs used in the treatment of RA are classified in two categories: drugs used for the symptomatic control of the disease (Corticosteroids and Non-steroidal anti-inflammatory drugs-NSAIDs) and disease modifying anti-rheumatic drugs (DMARDs) which are able to reduce disease progression (Radu and Bungau, 2021; Brown et al., 2024).

4.1. Symptomatic treatment of RA

The symptomatic treatment of RA is used only for the short-term management of inflammation and pain, consisting primarily of NSAIDs and corticosteroids. NSAIDs act by the inhibition of cyclooxygenases, with a subsequent reduction of prostaglandins level leading to an anti-inflammatory and peripheral analgesic effect. COX-2 selective NSAIDs (celecoxib, etoricoxib) can be used in order to minimize gastrointestinal adverse effects, but the risk of other unwanted effects like thrombotic events requires an assessment of the benefit-risk balance prior to their clinical use (Solomon et al., 2018; Brown et al., 2024).

Corticosteroids have a superior anti-inflammatory potency compared to the NSAIDs, due to their complex mechanism of action which includes the inhibition of arachidonic acid cascade but also the inhibition of several pro-inflammatory cytokines' genes. In RA, corticosteroids are used as a "bridging therapy" for DMARDs until the full development of their effects or associated with DMARDs as an adjunctive treatment (Radu and Bungau, 2021; Brown et al., 2024).

Corticosteroids (prednisone, prednisolone, dexamethasone) can be administered via multiple routes (oral, intravenous, intraarticular) but their use is generally limited in time (up to 3 months) by the development of significant adverse effects which may include hydro-saline retention, osteoporosis, diabetes, cataract, etc. (Singh et al., 2015; Radu and Bungau, 2021).

4.2. Disease modifying anti-rheumatic drugs (DMARDs)

DMARDs act by a variety of immunological mechanisms and are capable of altering the course of RA, promoting disease remission and delaying/stopping joint damage, dramatically improving patient's quality of life. They are classified as:

- conventional DMARDs
- biologic DMARDs
- targeted synthetic DMARDs (Brown et al., 2024).

4.2.1. Conventional DMARDs

Conventional DMARDs were introduced after 1970 and are considered to be the corner-stone of RA therapy, particularly for early or

mild forms of disease, due to their efficacy and large availability. Conventional DMARDs are chemically and pharmacologically diverse, methotrexate, leflunomide, sulfasalazine and hydroxychloroquine being the drugs of choice while d-penicillamine, azathioprine and gold salts are rarely used due to reduced efficacy and significant adverse effects (Radu and Bungau, 2021; Brown et al., 2024).

Methotrexate (MTX), a folate antagonist, is a first-line DMARD due to its efficacy in reducing disease activity and slowing radiographic progression (Brown et al., 2024). The international treatment guidelines recommend methotrexate as the first-line treatment of RA, generally as monotherapy for DMARDs-naïve patients, having better efficacy and a more rapid onset of action than other conventional DMARDs. In combination with a short bridging treatment with cortico-steroids, methotrexate can induce disease remission in approximately 40% of patients (Radu and Bungau, 2021). Associations with other molecules are possible in specific patients when therapeutic improvements are not achieved with MTX alone. Methotrexate inhibits dihydrofolate reductase, reducing DNA synthesis and lymphocyte proliferation, and exerts anti-inflammatory effects via adenosine release (Shinde et al., 2014). Administered mainly orally but also subcutaneously at 7.5–25 mg weekly, methotrexate achieves clinical improvement within 3–6 weeks, with one-third of patients showing radiological stability after 1 year. Adverse effects include hepatotoxicity (elevaited transaminases), leukopenia, and gastrointestinal upset, necessitating folic acid supplementation (1–5 mg daily) and regular monitoring of liver function and complete blood counts every 1–3 months (Visser and Van der Hejde, 2009; Wang et al., 2018).

Leflunomide reduces pyrimidine synthesis via the inhibition of dihydroorotate dehydrogenase, suppressing lymphocyte proliferation. Administered orally at a 100 mg loading dose for 3 days followed by 10–20 mg daily, it is effective in early RA, with clinical benefits within 4–6 weeks and efficacy comparable to methotrexate (Radu and Bungau, 2021; Brown et al., 2024). Adverse effects include diarrhea, hypertension, and hepatotoxicity (elevated transaminases), requiring monthly liver enzyme monitoring for the first 6 months (Alfaro-Lara et al., 2019).

Sulfasalazine, metabolized into sulphapyridine and 5-aminosalicylic acid, reduces inflammation via cyclooxygenase and lipoxygenase inhibition, resulting low concentration of prostaglandins. Dosed at 2–3 g daily, it achieves clinical effects within 6–10 weeks and is often used in early RA or as combination therapy (Brown et al., 2024). Adverse effects include gastrointestinal upset, rash, and hematologic abnormalities (leukopenia), particularly in slow acetylators. It is contraindicated in sulfonamide hypersensitivity or severe renal/hepatic impairment. Regular monitoring of blood counts and liver function is recommended (O'Dell et al., 2002; Brown et al., 2024).

Hydroxychloroquine, an antimalarial drug, modulates immune responses by inhibiting Toll-like receptors (TLRs) signaling and cytokine production. Dosed at 200–400 mg daily, it is used in mild RA or as part of combination therapy, with benefits evident after 2–3 months. It has a favorable safety profile, with rare adverse effects (retinal toxicity with long-term use), requiring annual ophthalmologic screening (Das et al., 2007; Brown et al., 2024).

4.2.2. Biologic DMARDs

Biologic DMARDs are an advanced therapeutic option available when conventional DMARDs are not sufficiently effective or they cause important adverse effects leading to poor patient acceptance. Biologic DMARDs are

generally obtained by recombinant DNA technology, and they target specific immune pathways, being indicated for moderate-to-severe RA, often combined with methotrexate to enhance efficacy (Radu and Bungau, 2021). Several studies have shown that a combined therapy of methotrexate and biologic DMARDs (infliximab, golimumab, adalimumab, etanercept, abatacept) showed an ACR50 response rate of 38% and a remission rate of 18% (Singh et al., 2015). Being directed against a variety of molecular targets, biologic DMARDs are subsequently classified according to the mechanism of action in:

- TNF-α inhibitors (infliximab, golimumab, adalimumab, certolizumb, etanercept)
- IL-1 inhibitors (anakinra)
- B-cell depleters (rituximab)
- Selective co-stimulation modulators (abatacept)
- IL-6 inhibitors (tocilizumab, sarilumab)

(Singh et al., 2015; Radu and Bungau, 2021; Brown et al., 2024).

TNF-α inhibitors act by blocking tumor necrosis alpha (TNF-α), a key proinflammatory cytokine, thus reducing synovial inflammation and joint destruction. Infliximab was the first molecule from its class to be authorized for the treatment of RA at the end of 1990s. It is a chimeric monoclonal antibody which binds to all forms of TNF-α, neutralizing its functions. Infliximab is dosed at 3 mg/kg i.v. every 8 weeks after loading doses and achieves clinical response in 50–60% of patients within 12 weeks (Van Vollenhoven, 2009; Van Vollenhoven, 2016). Golimumab is a fully human monoclonal antibody administered by s.c. or i.v. route at a dose of 50 mg every 4 weeks. It is a newer, better tolerated anti-TNF agent, compared to infliximab and etanercept (Pelechas et al., 2019). Adalimumab is a fully human monoclonal antibody, dosed at 40 mg s.c. every 2 weeks. It showed sustained efficacy over 5 years, with 60% of patients achieving low disease activity (Van de Putte, 2004). Etanercept is a fusion protein obtained by recombinant DNA technology by coupling the human TNF receptor p75 to an Fc fragment. It is dozed at 25 mg s.c. twice weekly or 50 mg weekly and reduces radiographic progression in 70% of patients (Emery et al., 2014). Certolizumab pegol is a PEGylated Fab fragment with long plasma T1/2, dosed at 200 mg s.c. every 2 weeks. It is effective in methotrexate-refractory RA, with rapid onset (2–4 weeks) and is approved also in pregnant women due to the lack of placental transfer (Bonek et al., 2021) (**Table 1**). IL-1 inhibitors are represented by Anakinra, an IL-1 receptor antagonist, dosed at 100 mg SC daily. It has modest efficacy (20–30% response rate) and is less commonly used due to frequent injections and injection-site reactions, being reserved for patients which are intolerant to other biological drugs (Mertens and Singh, 2009).

B-cell depleters are represented by rituximab, a chimeric monoclonal antibody which blocks CD20 epitope on B-cells, reducing their numbers. It is effective in the treatment of RA, as an alternative to anti-TNF agents and is not associated with a significant risk of infections. Rituximab is administered as an i.v. infusion, being particularly effective in seropositive patients (Porter et al., 2016).

The selective co-stimulation modulators are represented by abatacept, a fusion protein that inhibits T cell activation by binding to CD80 and CD86 molecules on the antigen presenting cells (APC), blocking their interaction with CD28 receptor on T cells. It is administered by i.v. route based on body weight (500–1,000 mg every 4 weeks). It reduces disease activity in 50% of metho-trexate-refractory patients, with a favorable safety profile (lower infection risk than anti-TNF agents) (Genovese et al., 2018).

Table 1. Main characteristics of DMARD drugs (Brown et al., 2024; Gao et al., 2024; McInnes and Schett, 2017; Radu and Bungau, 2021)

Class	**Drug**	**Mechanism of action**	**Route of administration**
Conventional DMARDs			
	Methotrexate	Inhibition of dihydrofolate-reductase	oral, s.c.
	Leflunomide	Inhibition of dihydroorotate-dehydrogenase	oral
	Sulfasalazine	Inhibition of nuclear factor kappa-B (NF-kB)	oral
	Hydroxychloroquine	Inhibition of immune activation by reducing TLR signaling and cytokine production	oral
Biologic DMARDs			
	Infliximab	Chimeric mAb against TNF α	i.v., s.c.
	Etanercept	Fusion protein which traps TNF α	s.c.
	Adalimumab	Human mAb against TNF α	s.c.
	Golimumab	Human mAb against TNF α	s.c.
	Certolizumab pegol	Pegylated humanized mAb against TNF	s.c.
	Anakinra	Antagonist of IL-1 receptor	s.c.
	Rituximab	Chimeric mAb against CD20 epitope	i.v.
	Abatacept	Fusion protein of CTLA4 and IgG1Fc	i.v., s.c.
	Tocilizumab	Humanized mAb against IL-6 R	i.v., s.c.
	Sarilumab	Human mAb against IL-6 R	s.c.
Targeted synthetic DMARDs			

	Tofacitinib	Inhibition of multiple JAKs	oral
	Baricitinib	Inhibition of multiple JAKs	oral
	Upadacitinib	Inhibition of JAK1	oral

Note: i.v. - intravenous; s.c. - subcutaneous; mAb - monoclonal antibody; R - receptor; TLR - Toll-like receptor; JAKs - Janus kinases

IL-6 inhibitors are represented by tocilizumab and sarilumab. Tocilizumab is an IL-6 re-ceptor antagonist, dosed at 8 mg/kg by i.v. route, every 4 weeks in patients with moderate-to-severe RA. It reduces inflammation and CRP levels within 2 weeks, with 50–60% of patients achieving remission (Navaro-Milan et al., 2012). Sarilumab is a recently developed drug from the same class, reserved to patients with uncontrolled form of RA (Fleischmann et al., 2017).

The safety profile of biologic DMARDs is superior to conventional drugs, severe adverse effects being rare. Injection site adverse effects were cited by several studies, but they were usually benign. A more significant adverse effect is the risk of serious bacterial or viral infections (Brown et al., 2024). Although the majority of infections in patients treated with biologic DMARDs are caused by common pathogens, in rare cases opportunistic infections were reported, reactivation of tuberculosis and B hepatitis being major concerns. The risk of serious infections also depends on patient's pre-existent comorbidities, use of corticosteroids and disease activity. Therefore, it is recommended that all patients should require a screening for tuberculosis and B hepatitis prior to the administration of biologic DMARDs (Salliot et al., 2009). No sufficient data were collected to present date to indicate if a particular drug from biologic DMARDs class carries an augmented risk of infections, compared to the other representatives. Although some reports have indicated an augmented risk of malignant melanoma in patients treated with TNF-α inhibitors, larger studies have not found any increase in melanoma risk in Europe (Mercer et al., 2017).

The authorization of biologic DMARDs has revolutionized the pharmacological therapy of RA, improving the patient's quality of life, but the high treatment cost poses a significant problem in developing nations. Thus, the recent development of biosimilars for several molecules from this class (infliximab, adalimumab, etanercept) has increased the use of biologic DMARDs in larger categories of patients with RA, worldwide (Brown et al., 2024; O'Neill et al., 2024).

4.2.3. Targeted synthetic DMARDs

The recently authorized drugs from the class of targeted synthetic DMARDs have a small molecular size, being able to act intracellularly by interfering specific cell signaling mechanisms. They are generally represented by Janus kinase inhibitors (tofacitinib, baricitinib, upadacitinib) and have the advantage of oral administration (Singh et al., 2016). Janus kinases family (JAK1, JAK2, JAK3, TYK2) are intracellular proteins located in a variety of immune cells, which act as signal transducers from cytokine signaling, being involved in the activation of transcription processes via JAK/STAT pathway, leading to an inflammatory response. Hence, the

inhibition of Janus kinases with small molecules exerts a significant favorable effect in autoimmune diseases like RA, limiting inflammatory reactions (Singh et al., 2016; Brown et al., 2024).

Tofacitinib and baricitinib are considered first generation drugs, inhibiting multiple types of JAKs while upadacitinib is a second-generation drug, inhibiting only JAK1. The American College of Rheumatology (ACR) guidelines published in 2021 recommend targeted synthetic DMARDs in patients with an inadequate response to conventional antirheumatic drugs. The available data did not show significant differences between biologic and targeted synthetic DMARDs concerning effectiveness in RA (Lauper et al., 2022).

Tofacitinib is a first-generation Janus kinase inhibitor, dosed at 5–10 mg orally twice daily which disrupts cytokine signaling, achieving remission in 30–40% of patients within 4 weeks (Lundquist et al., 2014). The newly authorized baricitinib and upadacitinib are less used in RA, several phase 4 studies being currently ongoing in order to investigate their safety profile (Radu and Bungau, 2021; Brown et al., 2024).

Adverse reactions for targeted synthetic DMARDs include upper respiratory infections, herpes zoster virus infections, hepatotoxicity, and hematologic abnormalities, requiring monitoring of liver enzymes and blood counts. Also, the rate of major adverse cardiovascular events (MACE) and malignancies was higher in patients treated with tofacitinib compared to anti-TNF compounds (Lundquist et al. 2014; Ytterberg et al., 2022).

5. International guidelines and treatment algorithms of RA

The main objectives of RA drug treatment are to achieve remission, preserve joint function, and minimize systemic complications with the ultimate goal of improving patients' quality of life. The international treatment guidelines of the European Alliance of Associations for Rheumatology (EULAR) from 2022 and of American College of Rheumatology (ACR) from 2021 recommend a treat-to-target approach, aimed at rapidly suppressing the inflammatory disease activity with cost-effective drugs which have also a good patient acceptance. The treatment's target would be either a significant remission or a reduction of disease activity evaluated by a disease activity score (DAS) which takes account of the affected joints (Fraenkel et al., 2021; Smolen et al., 2023).

In the early stages of rheumatoid arthritis, both EULAR and ACR guidelines recommend that the drug of choice should be methotrexate used at low doses, in newly diagnosed patients. A bridging short-term treatment with corticosteroids can be administered at the time of the initial diagnosis. Dose escalation protocols could be employed for methotrexate, or another conventional DMARD could be introduced, if needed (Fraenkel et al., 2021; Smolen et al., 2023; Gao et al., 2024).

In patients not reaching their treatment target with conventional drugs, a biologic/targeted synthetic DMARD should be introduced. ACR guidelines equally recommend biologic and targeted synthetic DMARDs, while EULAR guidelines specify that JAK inhibitors should be considered only after an evaluation of pertinent risk factors. Certain modifications of the international guidelines may exist at national level. In the United Kingdom, NICE guidelines recommend that failure of two conventional DMARDs is required ahead of the introduction of a biologic/targeted synthetic DMARD (NICE, 2018).

The choice of the initial biologic/targeted synthetic DMARD is a complex aspect, not very well clarified in the available guidelines. In case of an inadequate response to the initial

drug, subsequent biologic/targeted synthetic DMARDs can be introduced. There is evidence that in case of failure of response to a first TNF-α inhibitor, it is preferable to choose a second biologic DMARD with a different mechanism of action than use another anti-TNF agent (Emery et al., 2015).

The safety profile of a specific molecule and patients' comorbidities also plays a role in the process of DMARD selection. Thus, the potential adverse effects or contraindications could exclude certain treatment options in specific patients, according to the concept of "therapeutic matchmaking" recently introduced in the scientific literature (Konzett and Aletaha, 2024).

6. Natural compounds with antirheumatic properties

Alongside the authorized synthetic drugs used in the therapy of RA, a number of medicinal plants have in their chemical composition potential anti-arthritic constituents, with various mechanisms of action, which are generally well tolerated and could be used as adjuvants (Kaur et al., 2024). Several studies have demonstrated that specific natural compounds can alleviate rheumatic symptoms by targeting inflammatory pathways, offering a holistic approach to disease management, but further research is needed to ascertain their potential role in the therapy of RA.

Boswellic acid (BA), a terpenic compound from *Boswellia serrata* (Burseraceae) can reduce glycosaminoglycan degradation, which could maintain joint integrity and alleviate arthritis symptoms. In preclinical studies, BA reduced cartilage loss, exerting a beneficial role in synovitis, osteoarthritis and other joint disorders by inhibiting 5-lipo-oxygenase (5-LOX), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) and cyclo-oxygenase-2 (COX-2) (Safayhi et al., 1992). A meta-analysis of 7 clinical trials enrolling 545 patients with arthritis showed that the administration of Boswellia serrata extract for a mean period of 4 weeks reduced pain and improved joint mobility (Yu et al., 2020).

Quercetin (QU) is a flavonoid present in a variety of plant species and known for its anti-inflammatory properties. In a preclinical adjuvant-induced arthritis model in rodents, QU lowered the production of macrophage inflammatory mediators by regulating NF-kB activity and indirectly inhibiting angiogenesis and cartilage damage (Haleagrahara et al., 2017). In a clinical trial, the administration of quercetin 500 mg/day for 8 weeks in female patients with RA improved clinical symptoms like after-activity pain and morning pain (Javadi et al., 2017).

Epigallocatechin-3-gallate (EGCG), abundant in green tea (*Camellia sinensis*), could also serve as an effective adjunct therapy for RA. Preclinical studies showed that EGCG inhibited pro-inflammatory mediators like PGE2, COX-2, NF-κB and TNF-α (Ahmed, 2010). Also, EGCG suppressed autoimmune arthritis through the modulation of nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, according to another experimental study (Yu G et al., 2020). In a randomized controlled trial enrolling 50 patients with arthritis, a 4-week administration of green tea extract alongside an NSAID agent improved pain control and joint functions (Hashempur et al., 2018).

Peoniflorin, a terpenic glucoside present in paeony (*Paeonia lactiflora*) is a modulator of important intracellular pathways involved in inflammatory processes like MAPK and JAK/STAT, also reducing the level of TNF-alpha and IL-1 (Zhang and Wei, 2020). A meta-analysis of several randomized controlled trials enrolling 463 patients with RA showed

that the administration of total glycosides from peony for 12-24 weeks alongside methotrexate or leflunomide improved clinical outcomes, reducing also the intensity of the adverse effects of the conventional DMARDs (Huang et al., 2019).

Conclusions

Rheumatoid arthritis is a complex autoimmune disease requiring early treatment to achieve remission and prevent disability. Conventional DMARDs, particularly methotrexate, form the backbone of therapy, but newer biologic and targeted synthetic DMARDs are offering a superior control for refractory disease. Despite recent progress, challenges such as high treatment cost or the development of adverse effects warrant new research aimed at developing safer and more accessible therapies for rheumatoid arthritis.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Abhishek A, Nakafero G, Kuo CF, et al. (2018) Rheumatoid arthritis and excess mortality: down but not out. A primary care cohort study using data from Clinical Practice Research Datalink. Rheumatology Oxford 57(6):977-81. doi: 10.1093/rheumatology/key013.
2. Ahmed S (2010) Green tea polyphenol epigallocatechin 3-gallate in arthritis: progress and promise. Arthritis Res Ther 12(2):208. doi: 10.1186/ar2982.
3. Alfaro-Lara R, Espinosa-Ortega HF, Arce-Salinas CA (2019) Systematic review and meta-analysis of the efficacy and safety of leflunomide and methotrexate in the treatment of rheumatoid arthritis. Reumatol Clín 15(3):133-139. doi: 10.1016/j.reuma.2017.07.020.
4. Alivernini S, Firestein GS, McInnes IB (2022) The pathogenesis of rheumatoid arthritis. Immunity 55(12):2255-2270. doi: 10.1016/j.immuni.2022.11.009.
5. Bonek K, Roszkowski L, Massalska M, Maslinski W, Ciechomska M (2021) Biologic drugs for rheumatoid arthritis in the context of biosimilars, genetics, epigenetics and COVID-19 treatment. Cells 10(2):323. doi: 10.3390/cells10020323.
6. Brown P, Pratt AG, Hyrich KL (2024) Therapeutic advances in rheumatoid arthritis. BMJ 384:e070856. doi: 10.1136/bmj-2022-070856.
7. Cross M, Smith E, Hoy D, Carmona L, Wolfe F, Vos T, Williams B, Gabriel S, Lassere M, Johns N, Buchbinder R, Woolf A, March L (2014) The global burden of rheumatoid arthritis estimates from the global burden of disease 2010 study. Ann Rheum Dis 73(7):1316-22. doi: 10.1136/annrheumdis-2013-204627.
8. Das S, Padhan P (2017) An overview of the extraarticular involvement in rheumatoid arthritis and its management. J Pharmacol Pharmacother 8(3):81-86. doi: 10.4103/jpp.JPP_194_16.
9. Das SK, Pareek A, Mathur DS, Wanchu A, Srivastava R, Agarwal GG, Chauhan RS (2007) Efficacy and Safety of hydroxychloroquine sulphate in rheumatoid arthritis: A randomized, double-blind, placebo controlled clinical trial—An Indian experience. Curr Med Res Opin 23(9):2227-2234. doi: 10.1185/030079907X219634.
10. Emery P, Gottenberg JE, Rubbert-Roth A, et al. (2015) Rituximab versus an alternative TNF inhibitor in patients with rheumatoid arthritis who failed to respond

to a single previous TNF inhibitor: SWITCH-RA, a global, observational, comparative effectiveness study. Ann Rheum Dis 74(6):979-84. doi: 10.1136/annrheumdis-2013-203993.

11. Emery P, Hammoudeh M, Fitzgerald O, Combe B, Martin-Mola E, Buch MH, Krogulec M, Williams T, Gaylord S, Pedersen R, Bukowski J, Vlahos B (2014) Sustained remission with etanercept tapering in early rheumatoid arthritis. New England Journal of Medicine 371(19):1781-1792. doi: 10.1056/NEJMoa1316133.
12. Fleischmann R, van Adelsberg J, Lin Y, Castelar-Pinheiro GD, Brzezicki J, Hrycaj P, Graham NM, van Hoogstraten H, Bauer D, Burmester GR (2017) Sarilumab and Nonbiologic Disease-Modifying Antirheumatic Drugs in Patients With Active Rheumatoid Arthritis and Inadequate Response or Intolerance to Tumor Necrosis Factor Inhibitors. Arthritis Rheumatol 69(2):277-290. doi: 10.1002/art.39944.
13. Fraenkel L, Bathon JM, England BR, et al. (2021) American College of Rheumatology Guideline for the Treatment of Rheumatoid Arthritis. Arthritis Care Res (Hoboken) 73(7):924-39. doi: 10.1002/acr.24596.
14. Gao Y, Zhang Y, Liu X (2024) Rheumatoid arthritis: pathogenesis and therapeutic advances. Med Comm 5(3):e509. doi: 10.1002/mco2.509.
15. Genovese MC, Pacheco-Tena C, Covarrubias A, Leon G, Mysler E, Keiserman M, Valente RM, Nash P, et al. (2018) Longterm Safety and Efficacy of Subcutaneous Abatacept in Patients with Rheumatoid Arthritis: 5-year Results from a Phase IIIb Trial. J Rheumatol 45(8):1085-1092. doi: 10.3899/jrheum.170344.
16. Gibofsky A (2012) Overview of epidemiology, pathophysiology, and diagnosis of rheumatoid arthritis. Am J Manag Care 18:S295–S302.
17. Haleagrahara N, Miranda-Hernandez S, Alim MA, Hayes L, Bird G, Ketheesan N (2017) Therapeutic effect of quercetin in collagen induced arthritis. Biomed Pharmacother 90:38–46. doi: 10.1016/j.biopha.2017.03.026.
18. Hashempur MH, Sadrneshin S, Mosavat SH, Ashraf A (2018) Green tea (Camellia sinensis) for patients with knee osteoarthritis: a randomized open-label active-controlled clinical trial. Clin Nutr 37(1):85-90. doi: 10.1016/j.clnu.2016.12.004.
19. Huang Y, Wang H, Chen Z, et al. (2019) Efficacy and safety of total glucosides of paeony combined with methotrexate and leflunomide for active rheumatoid arthritis: A meta-analysis. Drug Des Devel Ther 13:1969–1984. doi: 10.2147/DDDT.S207226.
20. Javadi F, Ahmadzadeh A, Eghtesadi S, Aryaeian N, Zabihiyeganeh M, Rahimi Foroushani A, Jazayeri S (2017) The effect of quercetin on inflammatory factors and clinical symptoms in women with rheumatoid arthritis: a double-blind, randomized controlled trial. J Am Coll Nutr 36(1):9-15. doi: 10.1080/07315724.2016.1140093.
21. Kaur C, Mishra Y, Kumar R, Singh G, Singh S, Mishra V, Tambuwala MM (2024) Pathophysiology, diagnosis and herbal-medicine-based therapeutic implication of rheumatoid arthritis: an overview. Inflammopharmacology 32(3):1705-1720. doi: 10.1007/s10787-024-01445-8.
22. Konzett V, Aletaha D (2024) Management strategies in rheumatoid arthritis. Nature Reviews Rheumatology 20(12):760-769. doi: 10.1038/s41584-024-01169-7.
23. Lauper K, Iudici M, Mongin D, et al. (2022) Effectiveness of TNF-inhibitors,

abatacept, IL6-inhibitors and JAK-inhibitors in 31 846 patients with rheumatoid arthritis in 19 registers from the 'JAK-pot' collaboration. Ann Rheum Dis 81(10):1358-66. doi: 10.1136/annrheumdis-2022-222586.

24. Lundquist LM, Cole SW, Sikes ML (2014) Efficacy and safety of tofacitinib for treatment of rheumatoid arthritis. World J Orthop 5(4):504-511. doi: 10.5312/wjo.v5.i4.504.
25. McInnes IB, Schett G (2017) Pathogenetic insights from the treatment of rheumatoid arthritis. Lancet 389(10086):2328-2337. doi: 10.1016/S0140-6736(17)31472-1.
26. Mercer LK, Askling J, Raaschou P, et al. (2017) Risk of invasive melanoma in patients with rheumatoid arthritis treated with biologics: results from a collaborative project of 11 European biologic registers. Ann Rheum Dis 76(2):386-91. doi: 10.1136/annrheumdis-2016-209285.
27. Mertens M, Singh JA (2009) Anakinra for rheumatoid arthritis: a systematic review. J Rheumatol 36(6):1118-1125. doi: 10.3899/jrheum.090074.
28. Min SY, Yan M, Kim SB, Ravikumar S, Kwon SR, Vanarsa K, Kim HY, Davis LS, Mohan C (2015) Green tea epigallocatechin-3-gallate suppresses autoimmune arthritis through indoleamine-2,3-dioxygenase expressing dendritic cells and the nuclear factor, erythroid 2-like 2 antioxidant pathway. J Inflamm (Lond.) 12:53. doi: 10.1186/s12950-015-0097-9.
29. Navaro-Millan I, Singh JA, Curtis JR (2012) Systematic review of tocilizumab for rheumatoid arthritis: a new biological agent targeting the IL-6 receptor. Clin Ther 34(4):788-802. doi: 10.1016/j.clinthera.2012.02.014.
30. NICE Guideline NIfHaCE (2018) Rheumatoid arthritis in adults: management [NG100]. Downloaded from: https://www.nice.org.uk/guidance/ng100.
31. O'Dell JR, Leff R, Paulsen G, Haire C, Mallek J, Eckhoff PJ, Fernandez A, Blakely K, Wees S, Stoner J, Hadley S, Felt J, Palmer W, Waytz P, Churchill M, Klassen L, Moore G (2002) Treatment of rheumatoid arthritis with methotrexate and hydroxychloroquine, methotrexate and sulfasalazine, or a combination of the three medications: results of a two-year, randomized, double-blind, placebo-controlled trial. Arthritis Rheum 46(5):1164-70. doi: 10.1002/art.10228.
32. O'Neil LJ, Alpizar-Rodriguez D, Deane KD (2024) Rheumatoid arthritis: the continuum of disease and strategies for prediction, early intervention and prevention. Journal of Rheumatology 51(4):337-349. doi: 10.3899/jrheum.2023-0334.
33. Pelechas E, Voulgari P, Drosos AE (2019) Golimumab for rheumatoid arthritis. J Clin Med 8(3):387. doi: 10.3390/jcm8030387.
34. Picerno V, Ferro F, Adinolfi A, et al. (2015) One year in review: the pathogenesis of rheumatoid arthritis. Clin Exp Rheumatol 33(4):551–558.
35. Porter D, van Melckebeke J, Dale J, Messow CM, McConnachie A, Walker A, Munro R, McLaren J, et al. (2016) Tumour necrosis factor inhibition versus rituximab for patients with rheumatoid arthritis who require biological treatment (ORBIT): an open-label, randomized controlled, non-inferiority, trial. Lancet 388(10041):239-47. doi: 10.1016/S0140-6736(16)00380-9.
36. Radu A-F, Bungau SG (2021) Management of rheumatoid arthritis: an overview. Cells 10(11):2857. doi: 10.3390/cells10112857.
37. Safayhi H, Mack T, Sabieraj J, et al. (1992) Boswellic acids: novel, specific, nonredox inhibitors of 5-lipoxygenase. J Pharmacol Exp Ther 261(3):1143-1146.

38. Salliot C, Dougados M, Gossec L (2009) Risk of serious infections during rituximab, abatacept and anakinra treatments for rheumatoid arthritis: Meta-analyses of randomized placebo-controlled trials. Ann Rheum Dis 68(1):25-32. doi: 10.1136/ard.2007.083188.
39. Shinde CG, Venkatesh MP, Kumar TM, Shivakumar HG (2014) Methotrexate: a gold standard for treatment of rheumatoid arthritis. J Pain Palliat Care Pharmacother 28:351–358. doi: 10.3109/15360288.2014.959238.
40. Singh JA, Hossain A, Tanjong Ghogomu E, Mudano AS, Tugwell P, Wells GA (2016) Biologic or tofacitinib monotherapy for rheumatoid arthritis in people with traditional disease-modifying anti-rheumatic drug (DMARD) failure: a Cochrane Systematic Review and network meta-analysis (NMA). Cochrane Database Syst Rev 11(11):CD012437. doi: 10.1002/14651858.CD012437.
41. Singh JA, Saag KG, Bridges SL, et al. (2016) American College of Rheumatology guideline for the treatment of rheumatoid arthritis. Arthritis Care Res 68:1–25.
42. Smolen JS, Aletaha D, McInnes IB (2016) Rheumatoid arthritis. Lancet 388(10055):2023-2038. doi: 10.1016/S0140-6736(16)30173-8.
43. Smolen JS, Landewé RBM, Bergstra SA, et al. (2023) EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2022 update. Ann Rheum Dis 82(1):3-18. doi: 10.1136/ard-2022-223356.
44. Solomon DH, Husni ME, Wolski KE, Wisniewski LM, Borer JS, Graham DY, Libby P, Lincoff AM, Lüscher TF, Menon V, Yeomans ND, Wang Q, Bao W, Berger MF, Nissen SE (2018) PRECISION Trial Investigators. Differences in safety of nonsteroidal anti-inflammatory drugs in patients with osteoarthritis and patients with rheumatoid arthritis: a randomized clinical trial. Arthritis Rheumatol 70(4):537-546. doi: 10.1002/art.40400.
45. Van de Putte L.A, Atkins C, Malaise M, Sany J, et al. (2004) Efficacy and safety of adalimumab as monotherapy in patients with rheumatoid arthritis for whom previous disease modifying antirheumatic drug treatment has failed. Ann Rheum Dis 63(5):508-516. doi: 10.1136/ard.2003.013052.
46. Van Vollenhoven RF (2009) How to dose infliximab in rheumatoid arthritis: new data on a serious issue. Ann Rheum Dis 68(8):1237-1239. doi: 10.1136/ard.2009.111682.
47. Van Vollenhoven RF (2016) New therapeutic approaches in rheumatoid arthritis. Presse Med 45:179-192. doi: 10.1016/j.lpm.2016.05.004.
48. Visser K, van der Heijde D (2009) Optimal dosage and route of administration of methotrexate in rheumatoid arthritis: a systematic review of the literature. Ann Rheum Dis 68(7):1094-9. doi: 10.1136/ard.2008.092668.
49. Wang W, Zhou H, Liu L (2018) Side effects of methotrexate therapy for rheumatoid arthritis: A systematic review. Eur J Med Chem 158:502-516. doi: 10.1016/j.ejmech.2018.09.027.
50. Wu CY, Yang HY, Luo SF, Lai JH (2021) From rheumatoid factor to anti-citrullinated protein antibodies and anti-carbamylated protein antibodies for diagnosis and prognosis prediction in patients with rheumatoid arthritis. Int J Mol Sci 22(2):686. doi: 10.3390/ijms22020686.
51. Yu G, Xiang W, Zhang T, Zeng L, Yang K, Li J (2020) Effectiveness of Boswellia and Boswellia extract for osteoarthritis patients: a systematic review and meta-analysis.

BMC Complement Med Ther 20(1):225. doi: 10.1186/s12906-020-02985-6.

52. Ytterberg SR, Bhatt DL, Mikuls TR, et al. (2022) Cardiovascular and cancer risk with tofacitinib in rheumatoid arthritis. N Engl J Med 386(4):316-26. doi: 10.1056/NEJMoa2109927.
53. Zhang L, Wei W (2020) Anti-inflammatory and immunoregulatory effects of paeoniflorin and total glucosides of paeony. Pharmacol Ther 207:107452. doi: 10.1016/j.pharmthera.2019.107452.
54. Zhang Z, Gao X, Li S, Wang Q, Wang Y, Hou S, Wang J, Zhang Y (2025) Global, regional and national epidemiology of rheumatoid arthritis among people aged 20-54 years from 1990 to 2021. Sci Rep 15:10736. doi: 10.1038/s41598-025-92150-1.

Acta Biologica Marisiensis

PUBLIC KNOWLEDGE AND PERCEPTIONS OF PHYTOTHERAPEUTIC APPROACHES FOR ORAL CAVITY DISORDERS

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Abstract: Oral cavity disorders are frequently encountered among the general population. If left untreated they can produce serious health risks. Phytotherapy represents a valuable complementary approach in oral healthcare, with herbal remedies being widely used for different conditions. Despite their availability, the level of public knowledge regarding the safe and effective use of phytotherapeutic products remains underexplored. This study aimed to evaluate the knowledge, perceptions, and attitudes of the general population toward the use of phytotherapeutic treatments for oral cavity disorders. A cross-sectional survey was conducted among 178 respondents from the general population. Data were collected using a structured questionnaire assessing awareness of phytotherapeutic products, sources of information, perceived efficacy, and attitudes toward their use. Younger participants and those with higher education levels demonstrated significantly better awareness. Pharmacists (47.3%) are considered the most influential professional sources of recommendation. General attitudes were favorable, with 62.2% of respondents open to using herbal remedies as alternatives or complementary, and 65.7% recognizing their preventive potential. Phytotherapeutic products are perceived as safe, effective, and acceptable by the general population for the prevention and management of mild oral conditions. Their integration into oral care is best achieved under professional supervision, with emphasis on standardized formulations and evidence-based guidance.

Keywords: phytotherapy, oral cavity, public knowledge, pharmacist perception

1. Introduction

Oral health is an essential component of general well-being, with disorders such as gingivitis, stomatitis, oral candidiasis, and recurrent aphthous ulcers affecting a large number of the population worldwide. Conventional therapies often include antibiotics, antifungal, antiseptics, anti-inflammatory drugs, etc., that can be limited due to side effects or patient concerns about their safety (Vieira Colombo et al. 2016; Di Stefano et al. 2022; Shinkai et al. 2024). Complementary and/ or alternative therapies have gained an increased interest in the recent years. Phytotherapy has a long tradition in the

management of oral conditions and is frequently preferred due to the general impression that natural products are safer (Janakiram et al. 2020; Shinkai et al. 2024). There are several classes of phytochemicals used in herbal-based products used for different disorders. Herbal products that contain mucilage can be part of multicomponent preparations, associated with various other substances, because mucilages increase the contact time of these substances in the oral cavity, potentiating their effect. These combinations are useful in treating various conditions such as stomatitis, aphthous ulcers, etc. *Althaea officinalis*, *Malva sylvestris*, *Linum usitatissimum* and *Cetraria islandica* are often used as mucilage-containing herbal products (Miranda-Rius et al. 2015; Kręgielczak et al. 2023). Herbal products rich in tannins are well recognized for their astringent, antiseptic, and hemostatic properties. In oral healthcare, they are commonly applied in the management of stomatitis and gingivitis, particularly in the form of mouth rinses or gargles. Their therapeutic action is attributed to the ability of tannins to precipitate proteins on the surface of oral mucosa, thereby forming a thin protective film that protects the mucous membranes but also contributes to reducing gingival inflammation and controlling bleeding, while limiting microbial colonization and supporting tissue healing (Chandra Shekar et al. 2015; Ployon et al. 2018; Kováč et al. 2022). Several tannin-containing plants are traditionally used in oral care products: *Quercus robur*, *Camellia sinensis*, and *Punica granatum* (Venkateswara et al. 2011; Dabholkar 2016; Ștefănescu et al. 2022). Another important class of natural products are essential oils, with proven antimicrobial, anti-inflammatory, analgesic, and antioxidant properties, making them suitable for the prevention and management of various oral conditions. Their volatile compounds, such as terpenes, phenols, and aldehydes, interfere with microbial cell membranes, inhibit biofilm formation, and modulate local inflammatory responses. In dentistry and oral care, essential oils are most frequently used as components of mouth rinses, gels, or toothpastes, with applications in halitosis, gingivitis, periodontitis, dental plaque control, and mucosal infections. Some of the most popular essential oils include clove, peppermint, tea tree, thyme, cinnamon, and oregano (Kavanaugh and Ribbeck 2012; Marchese et al. 2016; Kerekes et al. 2019; Haro-González et al. 2021). However, despite their widespread use, public knowledge regarding the appropriate application, dosage, potential interactions, and safety concerns of phytotherapeutic treatments remains insufficiently studied. Misconceptions and reliance on non-professional sources of information may compromise treatment outcomes and there could be an increased risk to patient safety. Evaluating the population's knowledge and perceptions is therefore crucial in identifying educational needs and guiding healthcare professionals in providing evidence-based recommendations. The present study aims to assess the awareness, knowledge, and attitudes of the general population regarding the use of herbal-based treatments for oral cavity disorders. By analyzing socio-demographic influences and sources of information, this research wants to highlight existing knowledge gaps and provide a foundation for targeted public health interventions in oral health care. Although public interest in phytotherapy has grown across Europe, most existing studies assessing perceptions and attitudes toward herbal products have been conducted in Western countries. There is a lack of data from the Eastern and Central European populations, where cultural traditions regarding herbal-based products remains strong. To our knowledge, no published studies have

systematically explored the general public's view regarding herbal-based products for oral health in Romania, making this study, one of the first to address this gap. The present study aims to add a novel perspective to the current body of literature.

2. Materials and Methods

A cross-sectional study was conducted using an online questionnaire distributed between December 2024 and February 2025. The distribution of the questionnaire was approved by the Ethics Committee of the George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Târgu Mureș (Approval No. 3414/21.11.2024). Participation was voluntary and anonymous. By completing the questionnaire, respondents provided informed consent for the processing of their data. No exclusion criteria related to profession or educational level were applied, in order to capture the perception of the general public as accurately as possible. The questionnaire was disseminated online without targeting a specific population subgroup. The questionnaire included 37 items, organized into 6 thematic sections, that are presented in **Table 1**. Most items were formulated as ordinal Likert-scale questions, allowing respondents to indicate their degree of agreement or frequency on a scale of up to seven points, typically with 3–5 response options. Several questions permitted multiple responses (checkbox format), while a smaller number were open-ended items, enabling participants to provide additional qualitative input.

Table 1. Structure of the questionnaire and types of data collected

No.	Section	Items	Collected data	Sample questions/ Format of responses
1	Sociodemographic data	1-5	age, gender, education, place of residence, and occupation	*,,What is your gender?''/* single choice
2	History of oral conditions and treatment behaviors	6-12	frequency and types of oral conditions, use of plant-based products, source of recommendation	*,,How often do you use herbal-based products for oral hygiene?''/* single choice
3	Perceived efficacy and safety	13-17	comparative evaluation versus conventional medicines, subjective perceptions of health improvement	*,,To what extent do you consider herbal products to be effective compared to conventional medical treatment?''/* single choice
4	Adverse effects and information preferences	18-24	negative experiences, trust in information sources, preferences regarding professional counseling	*,, What side effects, if any, have you experienced following the use of herbal products for oral health issues?''* / multiple choice & open-ended
5	General attitudes toward phytotherapy	25-36	comparative trust versus conventional products, intention to recommend, perceived effective products, suggestions	*,,Which medicinal plants do you consider to be the most effective for conditions affecting the oral cavity?''/* multiple choice
6	Open comments	37	additional suggestions or opinions	*,, Do you have any other suggestions or comments related to herbal therapy products that you would like to share?''* / open-ended

3. Results and discussions

3.1. Sociodemographic data

A total of 178 respondents participated in the study, including both adults and a small proportion of individuals under 18 years of age. Age emerged as a key variable in the analysis, as it allowed for the observation of potential differences between age groups and provided deeper insight into how this factor might influence respondents' opinions. The largest proportion belonged to the 18–24 years age group (39.3%), highlighting the predominant participation of young adults. The next most represented category was 25–34 years (20.8%), followed by respondents aged 45–54 years (16.9%).

With regard to gender distribution, women were strongly overrepresented, accounting for 83.0% of the sample, while men represented only 18.0%.

Educational level also varied: 44.4% of participants reported having a university degree, 21.3% had completed high school, and 18.5% held postgraduate qualifications. In terms of professional background, 56.7% of respondents were from non-medical fields, whereas 38.2% were affiliated with the medical sector. A smaller fraction reported working as freelancers.

Place of residence showed that 64.4% of participants lived in urban areas, while 35.4% resided in rural areas. This distinction is relevant, as urban–rural differences may influence not only lifestyle but also access to information and educational opportunities. These demographic characteristics provide essential context for interpreting participants' knowledge, attitudes, and behaviors regarding phytotherapy in oral healthcare. The sociodemographic data indicates that young, educated, and urban living respondents were more engaged in the study, which is consistent with findings from other surveys on complementary medicine, where younger and more educated groups often report greater interest in natural health solutions (Shinkai et al. 2024).

3.2. History of oral conditions and treatment behaviors

Regarding the most frequent oral disorders mentioned by the respondents, aphthous ulcers where the most common (41.9%), followed by gingival bleeding (40.9%), oral inflammation (23.7%), halitosis (21.5%), and dental infections (16.1%), with lower frequencies for oral candidiasis and herpes. These results are comparable to the findings reported by Cunha et al. and Alhindi et al., suggesting a similar prevalence pattern across different populations (Alhindi et al. 2019; Zimmer et al. 2024).

Regarding treatment behaviors, 48.3% of participants indicated that they had used phytotherapeutic products for oral disorders, while 51.7% had not. This relatively balanced distribution may reflect either a lack of awareness about plant-based products for oral care or a preference for conventional pharmaceutical treatments, which are more familiar to the general population. Interestingly, country-specific differences have been observed: for instance, a study conducted in the United States reported that only 12.6% of respondents had used plant-based products for oral conditions, highlighting potential cultural and healthcare-related influences on treatment choices (Abebe et al. 2011).

When asked about the conditions for which phytotherapeutic products were used, respondents most frequently mentioned aphthous ulcers (68.4%), followed by gingivitis (24.2%), halitosis (11.6%), and stomatitis (10.5%). In terms of product type, herbal teas (56.1%) were the most commonly employed, followed by tinctures (44.7%) and gels (36.0%). Less frequently, respondents reported the use of essential oils (21.9%) or herbal mouthwashes (0.9%). These findings suggest

that herbal teas are perceived as both effective and easily accessible options for maintaining oral health.

With respect to frequency of use, the majority of respondents reported using phytotherapeutic products only “sometimes” (33.9%) or “rarely” (33.3%), whereas only 11.1% reported “frequent” use, and a very small proportion indicated that they “always” used such products. This distribution may reflect a limited awareness of the potential benefits of phytotherapy in oral hygiene and disease management.

As sources of recommendation, the pharmacist was identified as the most influential source (47.3%), followed by friends (31.1%), medical professionals (20.3%), and social media (19.6%). These findings underscore the key role of pharmacists in guiding patient decisions, likely due to their accessibility and credibility in relation to drugs and food supplements. This reflects the trust that the respondents offer to pharmacists, although the general impression of pharmacists towards their profession is not the same. Pharmacists are seen as patient-centered communicators which is supported by recent nationwide surveys among community pharmacists, emphasizing their need for enhanced training in patient-centered communication strategies (Chang et al. 2000; Rusu et al. 2022; Al-Kubaisi et al. 2025). The significant influence of friends highlights the impact of personal experience and peer communication, while the lower ranking of physicians suggests either a preference for more accessible sources of advice or the perception that phytotherapeutic products do not require medical prescription. Automedication, however, raises important concerns due to misuse, inappropriate dosing, allergic reactions, side effects and drug-herb interactions. Even for well-known plant species, severe consequences can be seen when the treatment is not supervised. Extracts of sage, chamomile, or licorice, for example, administered internally, may potentiate anticoagulant effects or interfere with metabolic pathways, increasing the risk of unwanted systemic consequences, while herbal drugs with a high tannin content or mucilage content can decrease the absorption of other drugs, if administered internally but also when applied topic, on the oral mucosa (Hu et al. 2005; Izzo 2005; Fasinu et al. 2012).

3.3. Perceived efficacy and safety

When asked to evaluate the efficacy of phytotherapeutic products compared with conventional treatments, the majority of respondents expressed a positive perception. Specifically, 57.2% considered such products effective, and 12.0% very effective, reflecting positive personal experiences. A further 28.3% rated them as neutral, suggesting either insufficient exposure or lack of confidence due to limited knowledge. Only 2.4% of participants perceived them as ineffective, which may be explained by expectations of a faster therapeutic effect or a stronger preference for pharmaceutical alternatives. With respect to oral health outcomes, 64.4% of respondents reported perceivable improvements following the use of phytotherapeutic products. In contrast, 26.8% were unsure, which may reflect occasional or inconsistent use, or difficulties in attributing observed benefits to the product itself. Reported beneficial effects included reduction of inflammation (54.2%), improved oral hygiene (38.9%), fresher breath (29.9%), and reduced dental sensitivity (25.7%) (**Fig. 1.**). Only a small minority reported no significant change, suggesting that herbal products are generally perceived as supportive adjuvants in maintaining oral health.

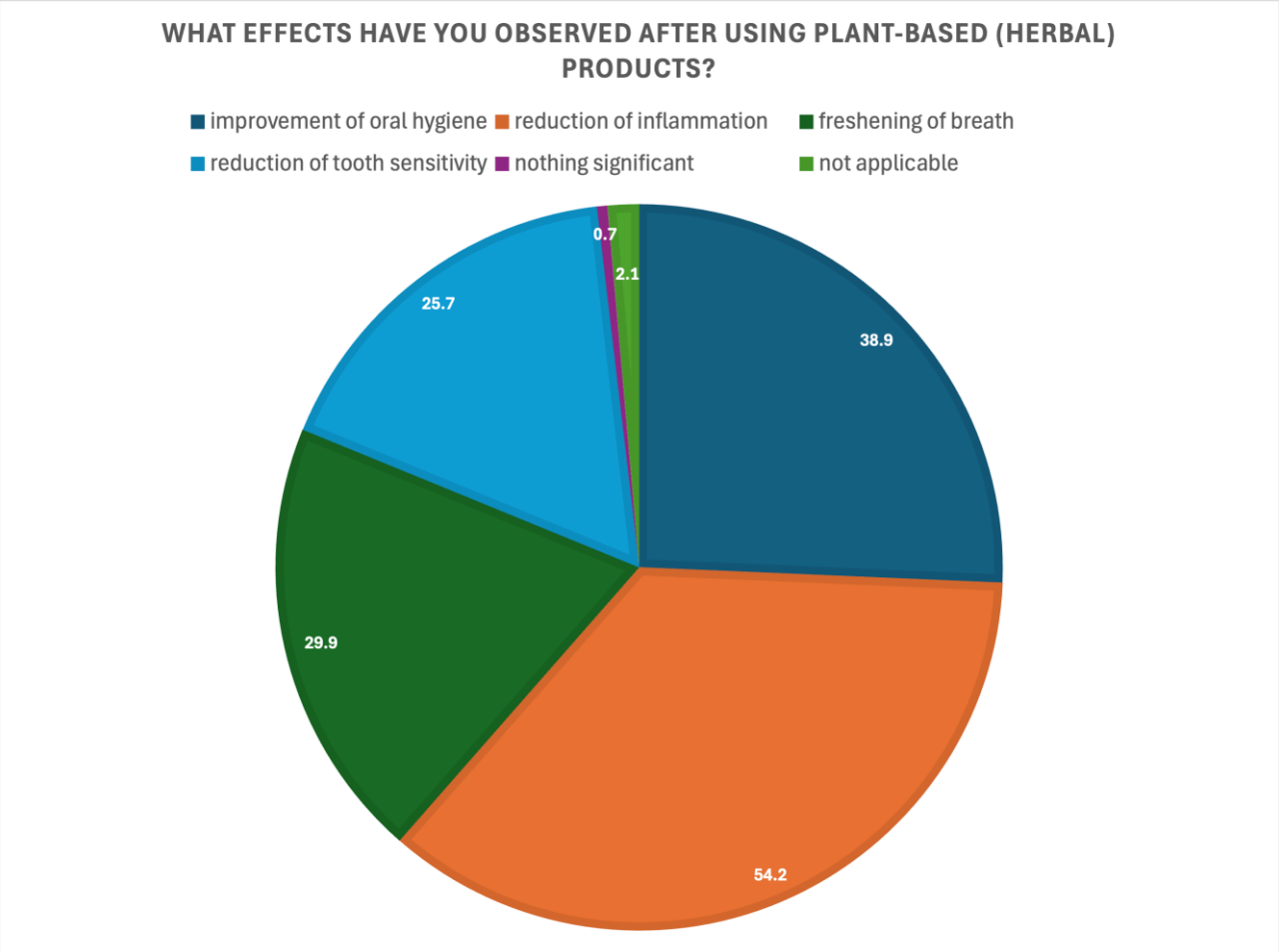

Fig. 1. Participants' responses on the effects of herbal products (multiple choice question)

When asked about the main advantages of phytotherapeutic products, respondents highlighted natural ingredients (60.2%), absence of side effects (46.0%), efficacy in treating oral problems (39.1%) and antimicrobial properties (39.1%). These responses emphasize the role of "natural origin" and perceived safety as strong factors for use.

In terms of safety perception, most participants considered phytotherapeutic products to be safe (53.3%) or very safe (22.8%), while 19.2% remained neutral. Only a very small proportion regarded them as unsafe. This perception over the safety profile of natural products increases the risk towards self-medication, and can lead to common mistakes like either therapeutic inefficiency or to the appearance of side effects, overdosing, etc (Hussain 1999; Consolini and Ragone 2010; Fainzang 2014).

3.4. Adverse effects and information preferences

The vast majority of respondents (95.5%) reported no adverse reactions following the use of phytotherapeutic products for oral conditions, while only 4.5% experienced negative effects. Among these, the most frequently mentioned were oral discomfort (21.1%), allergic reactions (15.8%), and local irritation (15.8%). The low incidence and mild nature of these events indicate that phytotherapeutic products are generally well tolerated and considered safe. Nevertheless, the occurrence of such reactions underscores the importance of proper labeling, awareness of product composition, and cautious use among individuals with known allergies.

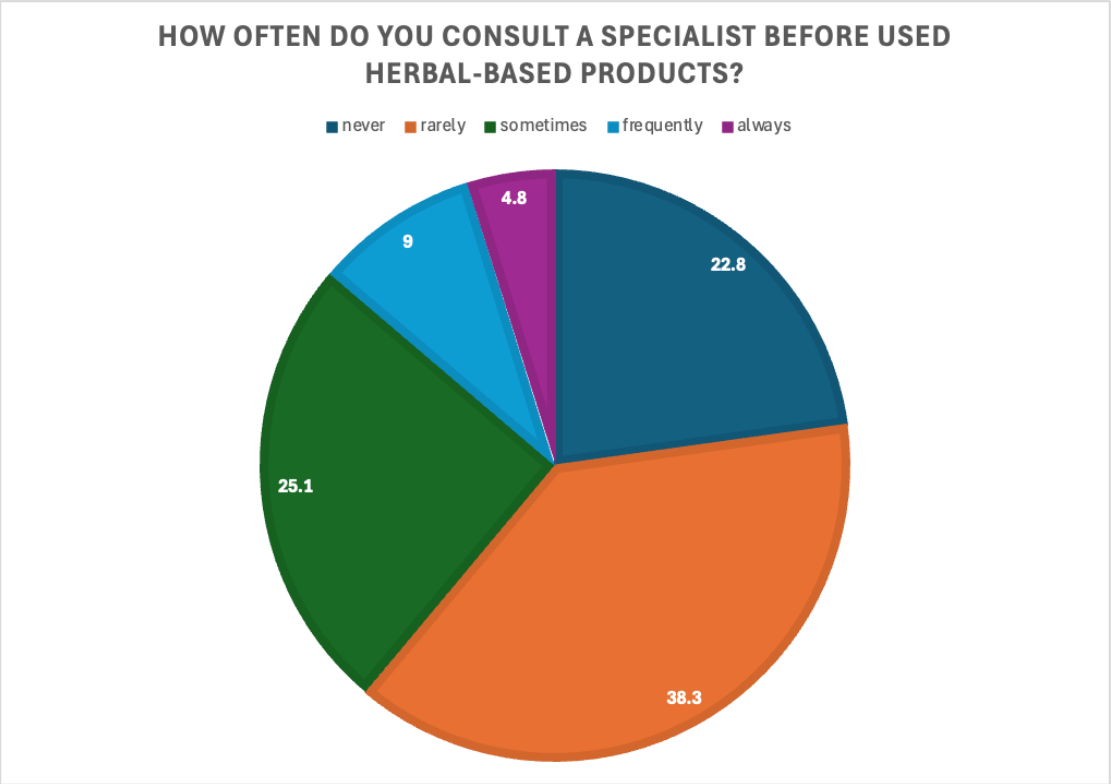

Fig. 2. Consultation frequency prior to using plant-based products

When asked about their willingness to receive professional guidance on phytotherapeutic products for oral health, the majority (86.6%) expressed a positive attitude, reflecting openness to professional advice and a preference for safe, informed use. However, 13.4% indicated no interest in specialist counseling, which may suggest limited awareness of potential risks or a tendency toward self-medication (Hussain 1999; Izzo 2005; Fainzang 2014). Regarding consultation practices, a considerable proportion of respondents admitted that they asked for professional advice rarely (38.3%), sometimes (25.1%), or never (22.8%). Only a small minority asked specialists frequently (9.0%) or always (4.9%) (**Fig. 2.**). These results suggest that many individuals perceive herbal products as mainly harmless and therefore not requiring professional supervision. Trust in dental professionals was also assessed. 41.9% of respondents reported a high level of trust in dentists' recommendations, while 45.9% expressed moderate trust. Lower trust levels were reported by 9.3%, and only a negligible fraction indicated complete lack of trust. Similar studies have found that a majority of adults demonstrate trust in their dentists and broader reviews indicate that trust leads to better patient engagement, reduced anxiety and the patients will obtain an improved oral health outcome (Armfield et al. 2017; Song et al. 2020).

3.5. General attitudes toward phytotherapy

The overall perception of respondents toward phytotherapeutic products for oral health was predominantly positive. When asked to compare their effectiveness with conventional medicines, 62.2% of participants answered "Yes," indicating openness to using herbal remedies either as an alternative in mild cases or as complementary therapy. These results are in accordance with the results obtained in clinical trials, that herbal-based treatments have a comparable efficiency in oral

health care with conventional therapy (Janakiram et al. 2020; Chatzopoulos et al. 2022). A smaller proportion (12.8%) expressed skepticism, reflecting a preference for conventional treatments and a perception that herbal products cannot substitute them. Meanwhile, 25.0% selected "I don't know," suggesting a significant proportion of respondents remain undecided, likely due to limited knowledge or insufficient personal experience.

Regarding safety, 42.4% of respondents perceived herbal products to be safer than conventional treatments, likely because of their natural ingredients and the belief that they cause fewer side effects. By contrast, 22.7% disagreed, possibly due to personal experiences with allergic or unwanted reactions. Interestingly, 42.4% responded "I don't know," indicating widespread uncertainty about the risk–benefit profile of phytotherapy. The factors most strongly influencing respondents' willingness to use phytotherapeutic products were medical recommendation (84.7%), followed by perceived efficacy (45.9%), and recommendations from family or friends and lower cost (14.4%). These results confirm the central role of healthcare professionals in shaping patient decisions, even for non-prescription natural products. Respondents also identified specific medicinal plants considered most effective for oral conditions. The most frequently chosen were chamomile (67.1%), propolis (61.7%), mint (46.7%), and sage (46.1%), all traditionally known for their anti-inflammatory, antiseptic, and soothing properties. Other popular options included aloe vera (54.5%) and echinacea (19.8%), suggesting openness to more recently promoted or "modern" herbal therapies. The influence of traditional medicine is evident, although global trends are also shaping preferences, as shown by U.S. studies highlighting species such as green tea, garlic, echinacea, *Ginkgo biloba*, and ginseng (Abebe et al. 2011).

In terms of information-seeking behavior, responses were almost evenly divided between those who actively searched information about medicinal plants (51.2%) and those who did not (48.8%). This balance suggests a growing but not yet universal public interest in the scientific validation of phytotherapy. When asked about their primary concerns when choosing herbal products, respondents prioritized efficacy first, followed by safety, then cost, while availability was the least concerning factor, indicating that these products are generally easy to access.

An open-ended question invited respondents to suggest ways to improve the quality and effectiveness of phytotherapeutic products. The most frequent recommendations included ensuring the purity of natural ingredients, conducting rigorous clinical studies, and providing professional guidance from physicians, pharmacists, or other health specialists. Concerns about ingredient authenticity and contamination are well-founded. Quality control is a recognized challenge in herbal medicine: inconsistencies in sourcing, processing, and misidentification of herbs can compromise both safety and therapeutic efficacy (Janakiram et al. 2020; Kim 2021; Wang et al. 2023). Other suggestions focused on improving the taste, formulation, and accessibility of products, highlighting user experience as an important factor in acceptance. Respondents also expressed preferences for diversification of herbal oral care products, citing interest in toothpastes, mouth rinses, gingival gels or ointments, breath sprays, whitening products, essential oils, and herbal gums or lozenges. This openness indicates strong consumer demand for a broader range of natural oral healthcare options.

While the present findings offer valuable insight into the perception and acceptance of herbal products for oral health, several limitations must be acknowledged. There is a demographic imbalance with a high number of female respondents and an overrepresentation of young adults. This difference in gender and age distribution limits the generalizability of the results to the broader population, especially older adults and male users, whose perceptions and behaviors may differ. Another limitation of the study is that it was not formally validated prior to distribution. Further research should aim to develop and validate a standardized instrument that could be applied across different populations and cultural settings.

Conclusions

This study highlights the generally positive perception of the general population regarding the use of natural products for oral health. A large proportion of respondents reported using or being willing to use herbal remedies, particularly for common conditions such as aphthous ulcers, gingival bleeding, and halitosis. Phytotherapy was perceived as effective and safe, with very few adverse effects reported, supporting its role as a complementary approach in oral healthcare. Trust in healthcare professionals, especially pharmacists, was identified as the most influential factor in guiding the decision to adopt herbal treatments, underscoring the importance of professional counseling in ensuring the safe and appropriate use of these products. Despite the positive attitudes, a significant proportion of respondents expressed uncertainty, reflecting gaps in knowledge and the need for more reliable information. This finding aligns with international evidence stressing the necessity of standardized formulations, clinical validation, and a clear evaluation of benefits and risks. In conclusion, natural products can be considered as a supportive strategy for maintaining oral health, particularly in mild and preventive contexts. Future efforts should focus on strengthening public education, integrating evidence-based herbal approaches into dental and pharmaceutical counseling, and promoting rigorous research to validate the efficacy and safety of commonly used plant-derived products.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Abebe W, Herman W, Konzelman J. 2011. Herbal supplement use among adult dental patients in a USA dental school clinic: prevalence, patient demographics, and clinical implications. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 111(3):320–325. https://doi.org/10.1016/j.tripleo.2010.10.012
2. Alhindi N, Sindi A, Binmadi N, Elias W. 2019. A retrospective study of oral and maxillofacial pathology lesions diagnosed at the Faculty of Dentistry, King Abdulaziz University. CCIDE. Volume 11:45–52. https://doi.org/10.2147/CCIDE.S190092
3. Al-Kubaisi KA, Abdel-Qader DH, Al Mazrouei N, Al-Azayzih A, Beshir SA,

Elnour AA. 2025. Community pharmacists' perception of knowledge, attitudes, and practices toward oral health promotion: A Cross-sectional study on the influence of training, demographics, and resources in the UAE.Wilkinson J, editor. PLoS One. 20(6):e0323193. https://doi.org/10.1371/journal.pone.0323193

4. Armfield J, Ketting M, Chrisopoulos S, Baker S. 2017. Do people trust dentists? Development of the Dentist Trust Scale. Australian Dental Journal. 62(3):355–362. https://doi.org/10.1111/adj.12514
5. Chandra Shekar B, Nagarajappa R, Suma S, Thakur R. 2015. Herbal extracts in oral health care - A review of the current scenario and its future needs. Phcog Rev. 9(18):87. https://doi.org/10.4103/0973-7847.162101
6. Chang ZG, Kennedy DT, Holdford DA, Small RE. 2000. Pharmacists' Knowledge and Attitudes toward Herbal Medicine. Ann Pharmacother. 34(6):710–715. https://doi.org/10.1345/aph.19263
7. Chatzopoulos GS, Karakostas P, Kavakloglou S, Assimopoulou A, Barmpalexis P, Tsalikis L. 2022. Clinical Effectiveness of Herbal Oral Care Products in Periodontitis Patients: A Systematic Review. IJERPH. 19(16):10061. https://doi.org/10.3390/ijerph191610061
8. Consolini A, Ragone M. 2010. Patterns of Self-Medication with Medicinal Plants and Related Adverse Events - A South American Survey. CDS. 5(4):333–341. https://doi.org/10.2174/157488610792246019
9. Dabholkar CS. 2016. Comparative Evaluation of Antimicrobial Activity of Pomegranate-Containing Mouthwash Against Oral-Biofilm Forming Organisms: An Invitro Microbial Study. JCDR [Internet]. [accessed 2025 Sep 10]. https://doi.org/10.7860/JCDR/2016/16478.7475
10. Di Stefano M, Polizzi A, Santonocito S, Romano A, Lombardi T, Isola G. 2022. Impact of Oral Microbiome in Periodontal Health and Periodontitis: A Critical Review on Prevention and Treatment. Int J Mol Sci. 23(9):5142. https://doi.org/10.3390/ijms23095142
11. Fainzang S. 2014. Managing Medicinal Risks in Self-Medication. Drug Saf. 37(5):333–342. https://doi.org/10.1007/s40264-014-0153-z
12. Fasinu PS, Bouic PJ, Rosenkranz B. 2012. An Overview of the Evidence and Mechanisms of Herb–Drug Interactions. Front Pharmacol [Internet]. [accessed 2025 Sep 10] 3. https://doi.org/10.3389/fphar.2012.00069
13. Haro-González JN, Castillo-Herrera GA, Martínez-Velázquez M, Espinosa-Andrews H. 2021. Clove Essential Oil (*Syzygium aromaticum* L. Myrtaceae): Extraction, Chemical Composition, Food Applications, and Essential Bioactivity for Human Health. Molecules. 26(21):6387. https://doi.org/10.3390/molecules26216387
14. Hu Z, Yang X, Ho PCL, Chan SY, Heng PWS, Chan E, Duan W, Koh HL, Zhou S. 2005. Herb-Drug Interactions: A Literature Review. Drugs. 65(9):1239–1282. https://doi.org/10.2165/00003495-200565090-00005
15. Hussain SH. 1999. Potential risks of health supplements – self - medication practices and the need for public health education. International Journal of Risk & Safety in Medicine. 12(3–4):167–171. https://doi.org/10.3233/JRS-1999-190
16. Izzo AA. 2005. Herb–drug interactions: an overview of the clinical evidence. Fundamemntal Clinical Pharma. 19(1):1–16.

https://doi.org/10.1111/j.1472-8206.2004.00301.x

17. Janakiram C, Venkitachalam R, Fontelo P, Iafolla TJ, Dye BA. 2020. Effectiveness of herbal oral care products in reducing dental plaque & gingivitis – a systematic review and meta-analysis. BMC Complement Med Ther. 20(1):43. https://doi.org/10.1186/s12906-020-2812-1
18. Kavanaugh NL, Ribbeck K. 2012. Selected Antimicrobial Essential Oils Eradicate *Pseudomonas* spp. and *Staphylococcus aureus* Biofilms. Appl Environ Microbiol. 78(11):4057–4061. https://doi.org/10.1128/AEM.07499-11
19. Kerekes EB, Vidács A, Takó M, Petkovits T, Vágvölgyi C, Horváth G, Balázs VL, Krisch J. 2019. Anti-Biofilm Effect of Selected Essential Oils and Main Components on Mono- and Polymicrobic Bacterial Cultures. Microorganisms. 7(9):345. https://doi.org/10.3390/microorganisms7090345
20. Kim N-C. 2021. Need for Pharmacopeial Quality Standards for Botanical Dietary Supplements and Herbal Medicines. Food Suppl Biomater Health. 1(1):e10. https://doi.org/10.52361/fsbh.2021.1.e10
21. Kováč J, Slobodníková L, Trajčíková E, Rendeková K, Mučaji P, Sychrová A, Bittner Fialová S. 2022. Therapeutic Potential of Flavonoids and Tannins in Management of Oral Infectious Diseases—A Review. Molecules. 28(1):158. https://doi.org/10.3390/molecules28010158
22. Kręgielczak A, Łukaszewska-Kuska M, Mania-Końsko A, Dorocka-Bobkowska B. 2023. Flaxseed (*Linum usitatissimum*), Chamomile (Matricariae flos) and Marshmallow (*Althaea officinalis*) Mouth Rinses in the Therapy of Oral Mucosa Diseases – A Review. Journal of Natural Fibers. 20(2):2228487. https://doi.org/10.1080/15440478.2023.2228487
23. Marchese A, Orhan IE, Daglia M, Barbieri R, Di Lorenzo A, Nabavi SF, Gortzi O, Izadi M, Nabavi SM. 2016. Antibacterial and antifungal activities of thymol: A brief review of the literature. Food Chemistry. 210:402–414. https://doi.org/10.1016/j.foodchem.2016.04.111
24. Miranda-Rius J, Brunet-Llobet L, Lahor-Soler E, Farré M. 2015. Salivary Secretory Disorders, Inducing Drugs, and Clinical Management. Int J Med Sci. 12(10):811–824. https://doi.org/10.7150/ijms.12912
25. Ployon S, Morzel M, Belloir C, Bonnotte A, Bourillot E, Briand L, Lesniewska E, Lherminier J, Aybeke E, Canon F. 2018. Mechanisms of astringency: Structural alteration of the oral mucosal pellicle by dietary tannins and protective effect of bPRPs. Food Chemistry. 253:79–87. https://doi.org/10.1016/j.foodchem.2018.01.141
26. Rusu A, Chereches MC, Popa C, Botezatu R, Lungu I-A, Moldovan O-L. 2022. Community pharmacist's perspective regarding patient-centred communication in conjunction with pharmaceutical practice: A cross-sectional survey. Saudi Pharmaceutical Journal. 30(9):1327–1344. https://doi.org/10.1016/j.jsps.2022.06.014
27. Shinkai RSA, Azevedo CL, De Campos TT, Michel-Crosato E, Biazevic MGH. 2024. Importance of phytotherapy for oral health care and quality of life in adults: A scoping review. Journal of Dental Sciences. 19(2):751–761. https://doi.org/10.1016/j.jds.2024.01.002
28. Song Y, Luzzi L, Brennan DS. 2020. Trust in dentist - patient relationships: mapping the relevant concepts. European J Oral Sciences. 128(2):110–119.

https://doi.org/10.1111/eos.12686
29. Ștefănescu R, Ciurea CN, Mare AD, Man A, Nisca A, Nicolescu A, Mocan A, Babotă M, Coman N-A, Tanase C. 2022. *Quercus robur* Older Bark—A Source of Polyphenolic Extracts with Biological Activities. Applied Sciences. 12(22):11738. https://doi.org/10.3390/app122211738
30. Venkateswara B, Sirisha K, Chava V. 2011. Green tea extract for periodontal health. J Indian Soc Periodontol. 15(1):18. https://doi.org/10.4103/0972-124X.82258
31. Vieira Colombo AP, Magalhães CB, Hartenbach FARR, Martins do Souto R, Maciel da Silva-Boghossian C. 2016. Periodontal-disease-associated biofilm: A reservoir for pathogens of medical importance. Microbial Pathogenesis. 94:27–34. https://doi.org/10.1016/j.micpath.2015.09.009
32. Wang H, Chen Y, Wang L, Liu Q, Yang S, Wang C. 2023. Advancing herbal medicine: enhancing product quality and safety through robust quality control practices. Front Pharmacol. 14:1265178. https://doi.org/10.3389/fphar.2023.1265178
33. Zimmer J, Garbin RR, Vogel MT, Rigo L. 2024. Prevalence of Oral Lesions Diagnosed at a Pathology Institute: A Four-year Analysis. Pesqui Bras Odontopediatria Clín Integr. 24:e230037. https://doi.org/10.1590/pboci.2024.004

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DYNAMIC ANALYSIS OF FORESTRY SECTOR IN ITALY: A LONG-TERM STATISTICAL ASSESSMENT WITH FAO DATA AND R LIBRARIES

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Abstract: Forestry plays the key role in environmental sustainability and economic wealth of Italy. Forest massifs maintain the ecological balance and climate setting of the country. At the same time, forests are essential sources of products for industry. The research objective is to evaluate the balance between the environmental protection and economic development of forestry. The research questions are: 1) how does forestry sector developing and what are the long-term trends showing its economic profitability? 2) How does the forest area change over decades? To answer these questions, the objective is to evaluate the data on environmental and economic parameters. The methodology included the FAOSTAT dataset on 2001-2023, processed by statistical analysis in R. The increase in forest area is detected from 12,748 to 13,937 T ha. Decline in pulp-and-paper sector is due to growing digitalization and e-commerce: newsprint production decreased from 293 T to 52 T tons, which proves transfer of printing industry towards e-format. Shifting market is in wrapping and packaging paper (360 T to 3.702 T tons). The results show positive trends in reforestation, high revenues from timber production which need further development and investments in forestry.

Keywords: economics; forestry; timber; pulp and paper; sustainable development; statistical analysis; R

1. Introduction

Forests have significant economic and ecological value for the country through providing marketable products (Farnworth et al., 1983; Adger et al., 1995; Bockstael et al. 2000) and delivering essential ecosystem services (Boyd & Banzhaf 2007; Zhang & Stenger, 2014; Shojaie shami et al., 2021). Forest massifs maintain the ecological balance of the country and serve as sources of products for forestry industry with major categories including timber, paper and pulp products. Essential environmental value of forest stands and vegetation consists in climate regulation (Winkel et al., 2011; Lemenkova, 2021; Yu et al., 2025), maintaining biodiversity (Raivio et al., 2001) and providing habitat spaces for rare and valuable species (Ruhl et al., 2020). Forest support healthy ecosystems through improved soil fertility and quality (Woo et al., 2025; Gaston et al., 2019), water purification and creating ecological habitats for rare and valuable species. Moreover, vegetation has important climateregulating function through mitigating temperature extremes carbon

sequestration and hydrological control on humidity (Keleş, 2018; Lemenkova 2025a; Li et al., 2010), hydrological regulating watershed level (Luo et al., 2025; Junttila et al., 2016; Lemenkova, 2022a; Fan et al., 2025).

Besides precious environmental functions, forests play important economic role in the society by providing goods of timber and non-timber origin to support livelihoods (Arts & Buizer, 2009). Economically, this includes direct and indirect use of products (e.g., fuel, wood, food, plants) originating from forest (Agrawal et al., 2008). Finally, forest provide intangible benefits for society through recreation and cultural services. Hence, high revenue from forests makes national forest policy decisions to balance between the environmental protection, which is crucial for nature conservation, and timber industries (Burnett & Davis 2002; Squire et al., 1991).

Such values of forest make them valuable resource for environmental protection agencies and economic purposes. Italy has a variety of tree species in forests that cover ca. 39% of the country. Major species include beech (*Fagus sylvatica*), oak (*Quercus*), and poplar (*Populus alba*). Poplar plantations, especially along the Po Valley, are a major source of feedstock for wood manufacturing sector. However, only a small percentage of forests of Italy is under detailed management for commercial timber, while the majority of the wood produced domestically is used as firewood, with industrial timber production declining. The decline is due to high costs, fragmented and poorly mechanized forestry sector, and strong dependence on imported raw materials.

As a result, forestry sector in Italy is under development and ongoing reconstruction. Currently it includes ca. 72,000 companies which employ 320,000 people. The forestry is characterized by a constantly growing forest area and a mix of modern and traditional equipment. Its major benefit of in Italy consists in the diversification, improving management plans and utilizing wood for structural and non-structural purposes. In 2022, the timber product consumption in Italy experienced a decline by 7.5% due to the pandemic, but then recovered and increased in demand for home improvement products of forestry sector. Nevertheless, the domestic roundwood production is insufficient for domestic needs, leading to significant dependance on import. Therefore, Italy imports a large part of the forestry sector for its needs, and remains a significant importer of timber products among the EU countries.

Commercial forest management in Italy faces challenges with major issues including small private property sizes, raw material shortage, limited transport and shipment capacities. Besides, despite a growing forest area, there is a low rate of timber harvesting compared to the amount grown. On the international level, there is a lack of forestry-related market balance in Italy, compared to other EU countries, e.g. Germany, Spain or France (Knauf, 2015). As a response to such problems, management of forestry industry in Italy is undergoing development. Regional policy in forestry and environmental sector now enhances environmental, social, and economic functions. These primarily include practices of sustainable management, supported by policies and funding, to ensure forest protection and production (Joyo et al., 2025). For instance, the EU Emissions Trading Scheme (EU ETS) brings positive effect by stimulating the pulp-and-paper making enterprises toward technological innovation (Lin et al. 2019). Recent studies using the Italian Community Innovation Survey (CIS) shown that the EU ETS exerted effects on environmental innovations (EI) in CO_2 abatement and energy efficiency controlling for other variables, grouped as internal and external to the firm, and additional

environmental regulation factors (Borghesi et al., 2015).

The fundamental concepts of the economic and environmental aspects of the forestry sector, timber and wood production are reflected in the economic aspects of Italy. An economic and environmental analysis of several case studies on forest products located in Italy was undertaken to analyze multiple products that can be obtained from forestry.

In the geographic sense, forest cultivation for industrial needs in Italy is well-adapted to the Mediterranean climate and has considerable ecological and economic potential. The main type of domestic wood used in the lumber industry of Italy is poplar, grown extensively in the Po Valley. Natural landscapes of forests represent the land surface where diverse environmental processes interplay to provide habitat and resources for life (Goldstein et al., 2002; Klaučo et al., 2013, 2017). Soil quality creates excellent conditions for plantation of forest massifs and wood production (Adams & Attiwill, 1984; Turvey & Smethurst, 1994; Imaya et al., 2005), which includes a variety of items: lumber, plywood, pulp-and-paper, engineered wood for construction and furniture (Lombardo, 2022). Understanding the benefits and demands in forestry sector remain a challenge since it involves the complexity of factors that affect forestry.

Although relevant literature on Italy exists, an integrated, systems-level appraisal of how these measures on environmental protection interact with economic benefits from forestry remains scarce. The studies on aspects of nature conservation seldom integrate industrial feedback loops from financial benefits of forestry, while papers on economical revenues from timber harvesting and wood processing frequently focus on technological aspects of market cycle in forestry, without reference to the environmental sustainability. Consequently, decisions in forest industry lack a holistic lens for prioritizing measures that would balance between forestry industry and environmental protection. Such measures would stimulate production of pulp-and-paper and timber for economic well-being, while taking measures on nature protection and conservation, such as development of network of the protected parks and natural reserves.

This research project uses statistical data available from the Food and Agriculture Organization (FAO) of the United Nations (UN). Existing applications of FAO in forestry and environmental research prove its robustness and applicability (Skulska et al., 2019). Spatial and descriptive data are employed from national services for regional approach focused on the analysis of forestry in Italy. It compares five sectors of forestry at regional level with varied situation on economic development in forest sector. We applied diverse workflows of R libraries for updating data and harmonization using combination of old data for retrospective analysis and novel data from the FAO-based survey to evaluate the period of 1961-2023. The qualitative-quantitative modelling of forestry development employed statistical methods of data processing by R programming and computing software to model economic trends, and spatial analysis by GIS. Important feature of R is provided by scripting techniques, which enable automating repetitive tasks and creating dynamic content in statistical computing of large datasets (Bilina & Lawford, 2012; Lemenkova, 2022b; Bröker et al., 2005). Available archived FAO datasets were processed by R statistical methods for accurate and effective visualization, to reveal trends in ecological and economic processes.

The importance of statistical data as a source of environmental and economic information can be illustrated by their practical application, including biodiversity monitoring (Sona et al., 2025), risk assessment (Jindal et

al., 2020; Lemenkova, 2024a), analysis of deforestation and aridification (Lemenkova, 2024b), environmental mapping, monitoring landscape dynamics, to mention a few.

Rapid development of the advanced statistical methods of data analysis presented effective modelling tools for dynamic updates for retrospective and perspective data analysis: R packages and libraries of Python (Chen, 2021; Hu et al., 2022; Lemenkova 2025b; Adun et al., 2021). Currently, there are no integrated studies that present an in-depth overview of both ecological and economic datasets that comparatively analyze the diversified landscapes and economic forestry sector of Italy. Using the advanced statistical methods for economic data processing, we performed the trend analysis of forestry products to demonstrate current situation and dynamics. Using advanced tools in R for studying environmental and economic variables is a powerful approach. Here, we use the RStudio for modelling FAO-based data.

This study contributes to this gap by quantifying a revenue from forestry and analyzing trends in production in 2 recent decades, and analyzing how forest stands increase in the pattern on land cover types in Italy. To achieve this goal, this study answers the following research questions: 1) how does forestry sector developing and what are the long-term trends showing its economic profitability? 2) How does the forest area change over decades? Answering these questions by R-based data analysis of FAO archives, this study provides insights into the variation between diverse environmental and economic parameters of forestry sector across the country, and their response to the environmental factors and trade affecting their dynamics – technologically, economically, and in ecological terms. This cumulative analysis of supported by statistical modelling in R aims to highlight the differences and variations in wood production and revenues and their changes over time for country level.

Research hypothesis of this study consists in the following statement: Forest industry in Italy will have higher impact factor on the economics due to the high revenues obtained from the furniture manufacturing and pulp-and-paper industry, provided the sustainable development of environmentally protected areas is supported through optimized land management.

This study develops a systematic approach to monitoring and comparative analysis of the forestry sector in Italy using advanced scripting tools of R libraries (Cribari-Neto & Zarkos, 1999). The specific aim is to model, analyze, and compare Italian landscape on forest industry using FAO data in 5 distinct categories: production quantity, import quantity and value, export quantity and value. The situation of forest trade was selected because forestry sector is rapidly growing in Italy, with forest area increasing by ca. 100,000 ha per year due to the abandonment of marginal agricultural land. The country has approximately 11.4 M ha of forest, covering about 39% of its territory. The significant portions of forestry are managed for timber production and conservation. The development of forestry in Italy is guided by a governmental support and includes initiatives for biodiversity protection and sustainable forest management.

Current state-of-the-art in research on Italian forestry has gap between the existing environmental applications and the advanced statistical methodologies. Based on literature analysis, the existing approaches of modelling dynamics in forestry sector in Italy are mostly relied on traditional methods with limited regional extent. This project aims to minimize this gap by developing appropriate methodologies by R for integrated extensive modelling using advanced tools and RStudio software. R is selected because it is a powerful

open-source programming language specifically designed for statistical analysis. Many case studies report the use of R in research on data modelling, visualization and analysis (Kleiber & Zeileis, 2008; Lemenkova, 2019a, 2019b; Anderson et al., 2018; Shahare et al., 2024). R provides a flexible and robust environment for data handling and statistical modeling, supported by a vast collection of libraries (Peabody, 2023; Choi & Asilkalkan, 2019). Its scripting nature allows for reproducible analysis, making it a crucial tool in environmental data science.

2. Materials and Methods

The Food and Agriculture Organization (FAO) data form a valuable information on environmental and economic variables that can be used for practical scientific purposes. The importance of such data is widely discussed in relevant works and can be summarized as follows:

1. First, statistical data present a key source of information on environmental, economic, climate and social-economic categories. It is a ground basis for modelling aimed at practical decision making on the environment protection, analysis of climate change and balance with economic development in forestry, including production of wooded materials.
2. Comparative analysis of economic and environmental categories enables to evaluate vulnerable sectors with rapidly changing trade rates to detect continuous process of economic development of country and to get insight into landscape dynamics.
3. Processing, interpreting, and visualising multi-format datasets using advanced statistical tools and software, such as R, improves interpretation of the datasets for statistical purposes.
4. Data interpretation provides information for the long-term economic prognosis. This is especially actual for forestry and wood production, since this sector should maintain the balance between forest protection and nature conservation on the one hand, and economic development on the other (production of wood and paper for export and regional needs).

The geospatial analysis of forest landscapes has been based on the QGIS to analyze the location of the protected forest stands in Italy and their proximity to the industrial centers (**Fig. 1.**).

With the advances of FAO mission and statistical data acquisition on forestry and environment, the amount of economic-geographic data that can be used for modelling is increasing progressively. This includes both open-source datasets and repositories, as well as data obtained from census, archives and official agencies for updates of economic and environmental analysis. Automated handling of these data for statistical analysis in environmental and economic monitoring remains a challenge. The solution to these tasks can be found using integration of data and computer-based processing through application of R statistical libraries. Technical algorithms of R use advance syntax which provides excellent instruments for in-depth environmental and economic analysis. Such integrative approach stands apart from the traditional descriptive analysis through methods of statistical visualization and analysis of diverse categories of data. Techniques included computed regression of the data, box plots, multi-facetted graphs, correlation plots and pie charts.

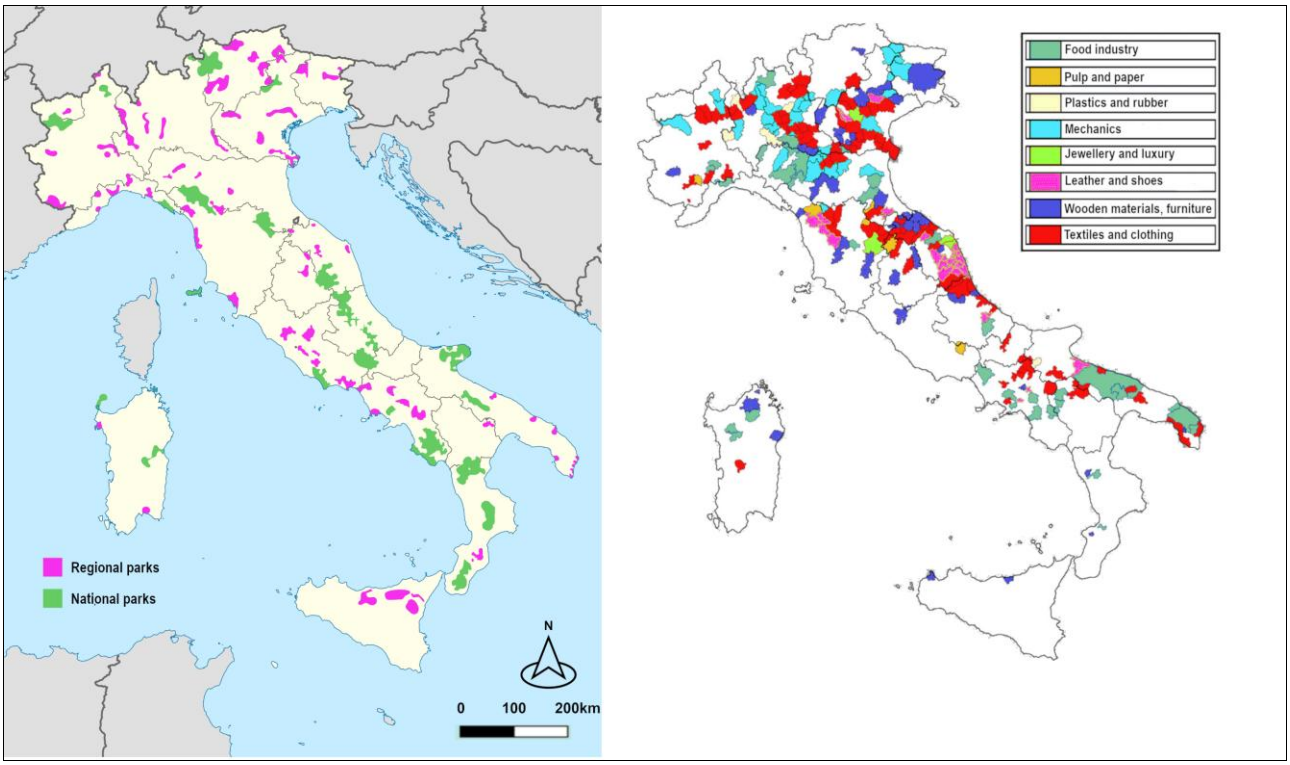

Fig. 1. Location of the protected forest stands in Italy (left). Source: Wikipedia, modified by the author; Industrial districts per sector in Italy (right) Source: ISTAT (2001), modified by the author.

The collected data were converted to tables with comma separated values (.csv format) and exported in into R. The important issue in forestry and environmental analysis is quantitative measure of trends in economic values of forestry products. This becomes the issue in regions with restricted wood and timber, such as Italy. To this end, we evaluated the dynamics over an extended period (1960 to 2023) covering a long-term period of six decades. Changes were interpreted using libraries of R (ggplot2 and others) to compare actual and retrospective data. This enabled to explain the distribution and structure of forestry sector, their dominant types and revenue for the country, changes and relation to forest protection, based on the statistical analysis. The methodology included statistical techniques of data analysis to evaluate perspective for forestry sector. In FAO repository, we selected the domain on "Forestry Production and Trade" in Italy (area code M49 380) with code "FO". The selected element with code 5516 covered data on forestry production.

The following categories of forestry sector were evaluated and assessed for economic analysis of trade and production: wood fuel (coniferous and non-coniferous); sawlogs and veneer logs, coniferous; pulpwood, (production 1961-2023, from coniferous and non-coniferous, diverse categories); other industrial roundwood, coniferous and non-coniferous (production); wood charcoal, chips, pellets and particles; recovered paper and paper production (diverse categories). Afterwards, the same categories were evaluated for export quantity (in m^3) and export value (in thousands $ USD), to analyze the revenue of forestry in the economic sector of the country and to evaluate trends and dynamics in monetary benefits from forestry industry in Italy.

The next step included the same categories of forestry production and trade with focus on import. This enabled to analyze the dependance of the country on imported products, their

values and price, and to compare the missing products in the economic system of the country. Although forestry sector in Italy includes a variety of companies, its domestic roundwood production is insufficient for domestic needs, which creates dependance on import of raw materials and finished products. This step highlighted the quantity and price of the imported goods in forestry.

The dynamics of land cover types was evaluated using remote sensing (RS) data of Moderate Resolution Imaging Spectroradiometer (MODIS) sensor (Barnes et al., 1998; Kahn & Sayer 2023). Evaluating the intensity of landscape dynamics using remote sensing (RS) data enables to reveal current state of the land cover types and analyze trends (Kocaman & Ağaçcıoğlu, 2025; Kesgin Atak & Tonyaloğlu, 2025; Lemenkova, 2025c; Chaves et al., 2025). RS data support comparison of ecosystem structure in its different areas, associated with climate factors. A sensor on NASA's Terra and Aqua satellites, MODIS collects RS data in 36 spectral bands, providing global coverage every one to two days which makes it reliable source of geo-information (Justice et al., 1998; Salomonson et al., 2006). On these data, changes of forest coverage were evaluated to highlight the environmental dynamics.

3. Results

The results demonstrated two issues: 1) environmental positive dynamics through reforestation trends in Italy with continuously increasing areas covered by forests; 2) the economic dynamics in forestry industry with a distinction on raw products: pulp and paper (newsprint, packaging paper, tissue and household paper, and paper for printing and writing), timber products (chips), wood and sawn wood. The rapidly developing forestry sector supplies products for economic industry and contributes to the country's well-being and sustainable development.

The evaluation of land cover types in Italy aimed at assessment of current trends in landscape dynamics according to distribution of land categories (in 1000 ha). The class “Artificial surfaces” (including urban and associated areas) demonstrated the rise in coverage from 1567,77 T ha in 2001 until 1587,51 T ha in 2023, which indicates a stable increase of the impervious surfaces due to urbanization. Variations in land cover types are shown in **figure 2**. The area occupied by herbaceous crops in Italy decreased from 11859,17 in 2001 until 10897,35 in 2023. Grasslands covered 3220,95 T ha in 2001, while 2023 their coverage slightly decreased to 3124,04 T ha, which proved the decline in ecosystems dominated by grasses. Forests and tree-covered areas, in contrast, increased from 12748,35 in 2001 to 13937,72 T ha in 2023, which proved their restoration at the country levels.

The Maquis shrubland, which is a shrub-covered area in Italy are presented by a dense vegetation of evergreen shrubs and small trees in coastal and Mediterranean regions. Their coverage declined significantly from 23,5 to 4,92 T ha. Such negative dynamics proves vulnerable state of landscapes, due to the climate warming, typical for southern regions. This type of ecosystem requires temperate or humid winters and hot, dry summers. Recent effects from climate change affected their distribution in the Mediterranean coasts. Shrubs or herbaceous vegetation, aquatic or regularly flooded are found in freshwater marshes, lagoons, and wetlands of Italy, such as the Padule di Fucecchio. These habitats feature communities like the *Phragmitetum communis* (common reed) and other aquatic and marsh plants, though the diversity can be impacted by factors like invasive species. This land cover type contrasts with other natural

vegetation types in Italy, such as coastal and inland shrubland (macchia), pastures, and grasslands. Their distribution declined from 284,73 T ha in 2001 to 201,67 in 2023, which proves the effects from climate and human activities (**Fig. 3.**).

Terrestrial barren land declined in Italy from 178,7 to 148,03 T ha, which correlates with the increase of areas in construction or natural regeneration of forests (e.g., the increase of forests and shrubland). Permanent snow and glaciers declined from 28,04 T ha in 2001 to 9,64 in 2023, according to MODIS satellite data. This concerns the mountain chains of Italy, such as Alps, Apennines and Dolomites.

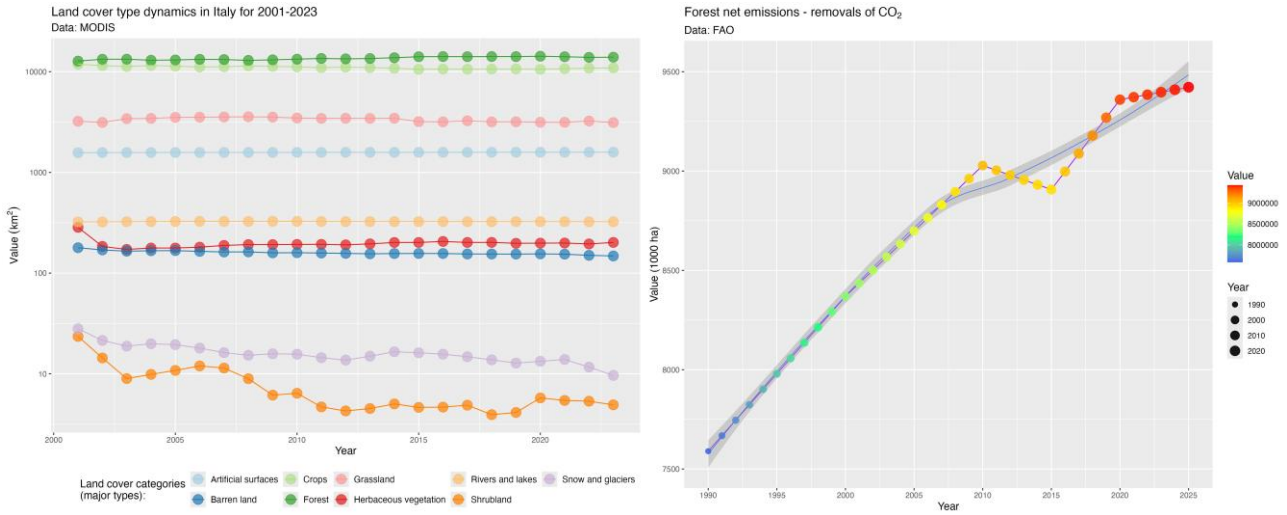

Fig. 2. Dynamics of land cover types in Italy (left) and forest emissions as CO_2 removals in 1000 ha (right). Data: FAO and MODIS. Software: R. Source: author.

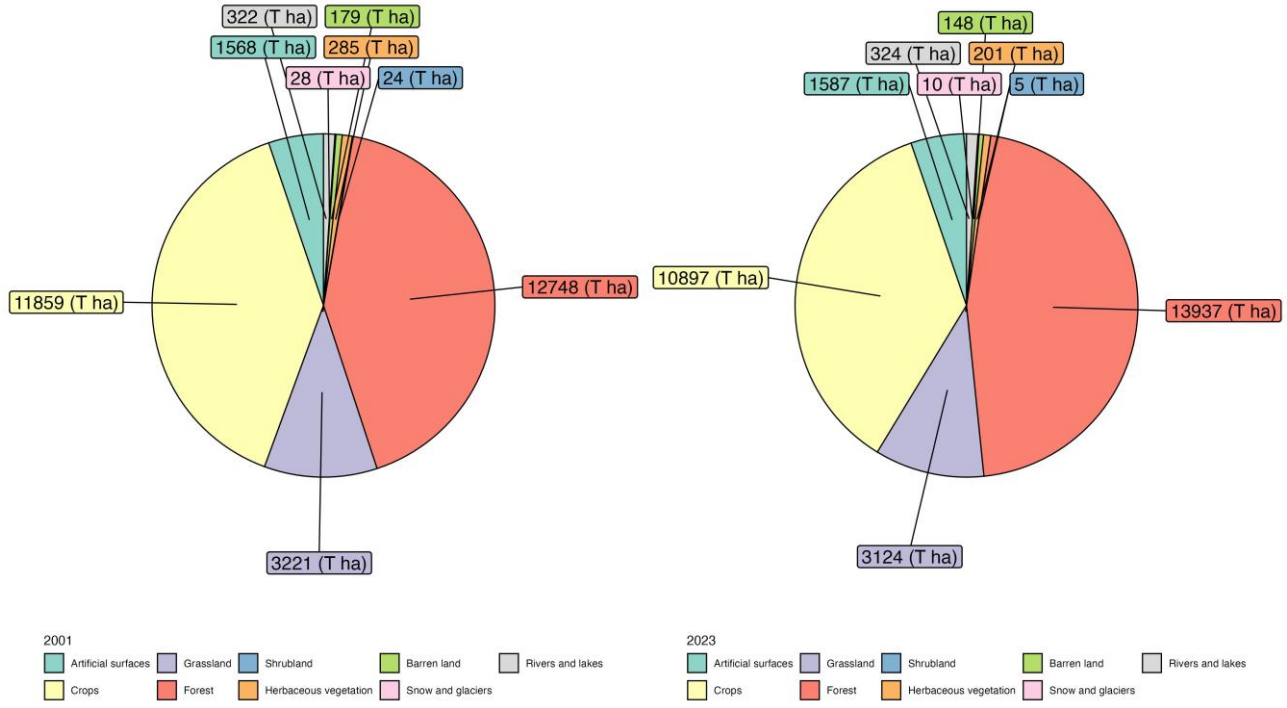

Fig. 3. Dynamics in share of land cover types (major categories) for Italy: 2001 (left) against 2023 (right). Data: MODIS. Software: R. Graph source: made by the author.

This proves gradual increase in temperatures that lead to the glacier and snow melt in the high Alpine areas. The category "Inland water bodies" did not experience significant changes during the evaluated period: in 2001, the inland water bodies occupied the area of 322,11 T ha, while in 2023 their coverage changed only a little, to 323,6 T ha. This indicates relatively stable situation of river network in Italy (**Fig. 3**).

In the forestry sector, numerous wood-based products are produced, such as recovered paper, wrapping of packaging, newsprint, roundwood, case materials, sawnwood, wood-based panels and cellulose and paper products for household papers. At the same time, pulp and paper industry is among the most energy-intensive industries which affect environment through increased industrial CO_2 emissions. Therefore, forestry sector and related pulp-and-paper industry of Italy followed the EU Emission Trading Scheme (ETS) directives which brings positive effects by stimulating the pulp-and-paper making enterprises toward technological innovations and less energy consumption. The pulp and paper industry strongly related to forestry sector and is currently accounts for about 6 % of the regional energy use.

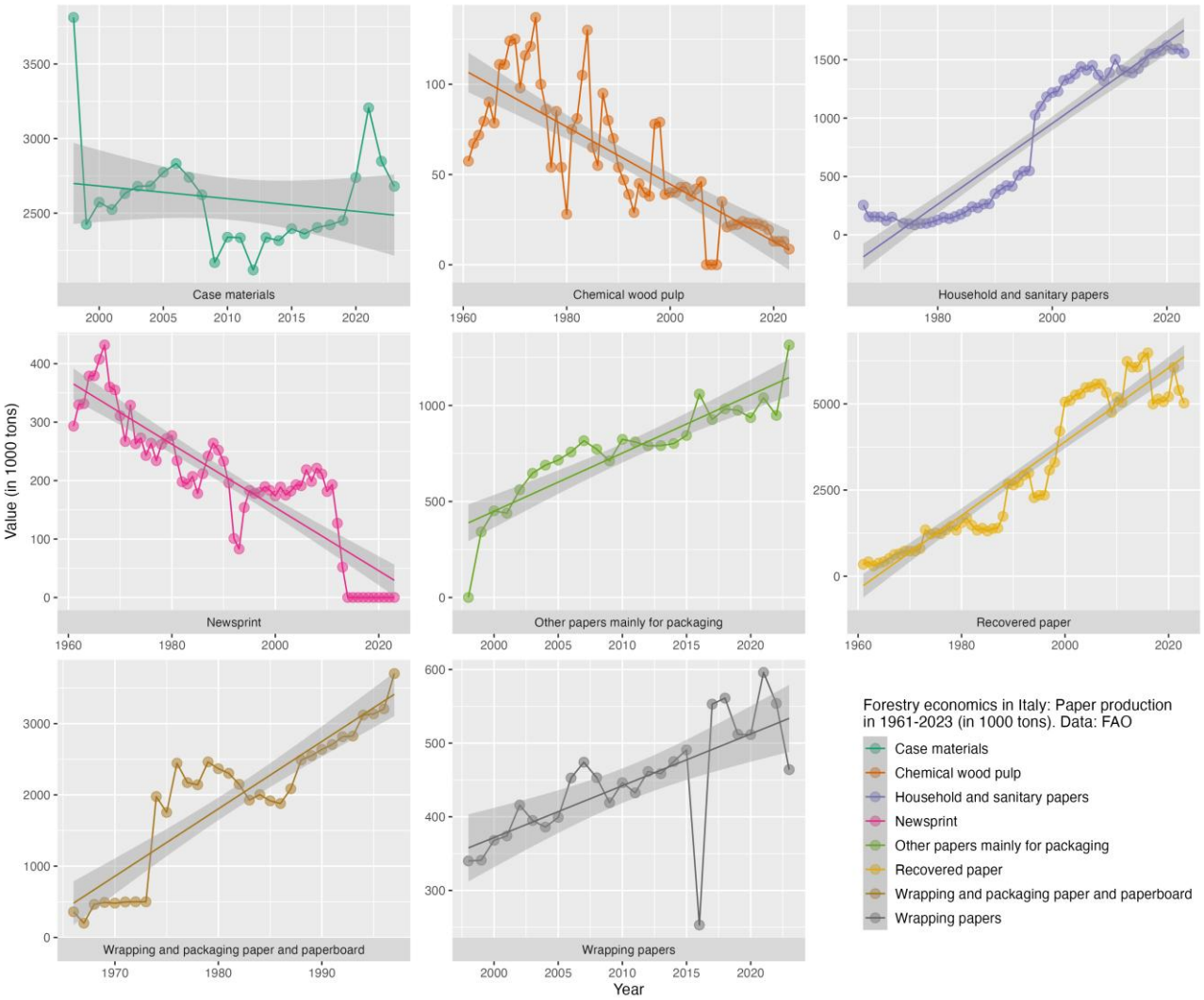

Fig. 4. Forestry economics in Italy: Paper production in 1961-2023 (in 1000 tons). Data: FAO. Software: R. Graph source: made by the author.

Newsprint production experienced decline from 293 T tons to 52 T tones in recent decades, due to the transfer of printing industry towards e-format. Other types of products, in contrary, increased: production of wrapping and packaging increased from 360 T tons to 3.702 T tons, household, case materials generally decreased from 3.811 T tons to 2.680 T tons, despite some fluctuations related to market demand. The recovered paper increased in volume from 344 T tons to 5.022 T tons. Such market dynamics was estimated in metric tons and indicated the development of forestry production and trade section. The data on pulp production were investigated in detail with analyses of the national wood fiber balances for the pulp and paper sector for recent decades (**Fig. 4.**)

The next part of the analysis compares the major export products in wood fiber and pulp-and-paper input (including produced and recovered paper), wrapping paper and paperboard sector with the export output of this sector (**Fig. 5**). The most significant data inputs were identified: 'recovered paper', followed by 'other paper and paperboard' with diversified items, and 'printing and writing papers'. the analysis also determined the minimal input categories, such as 'newsprint' which experienced shift towards the e-based platforms of newspapers both globally and at the regional level.

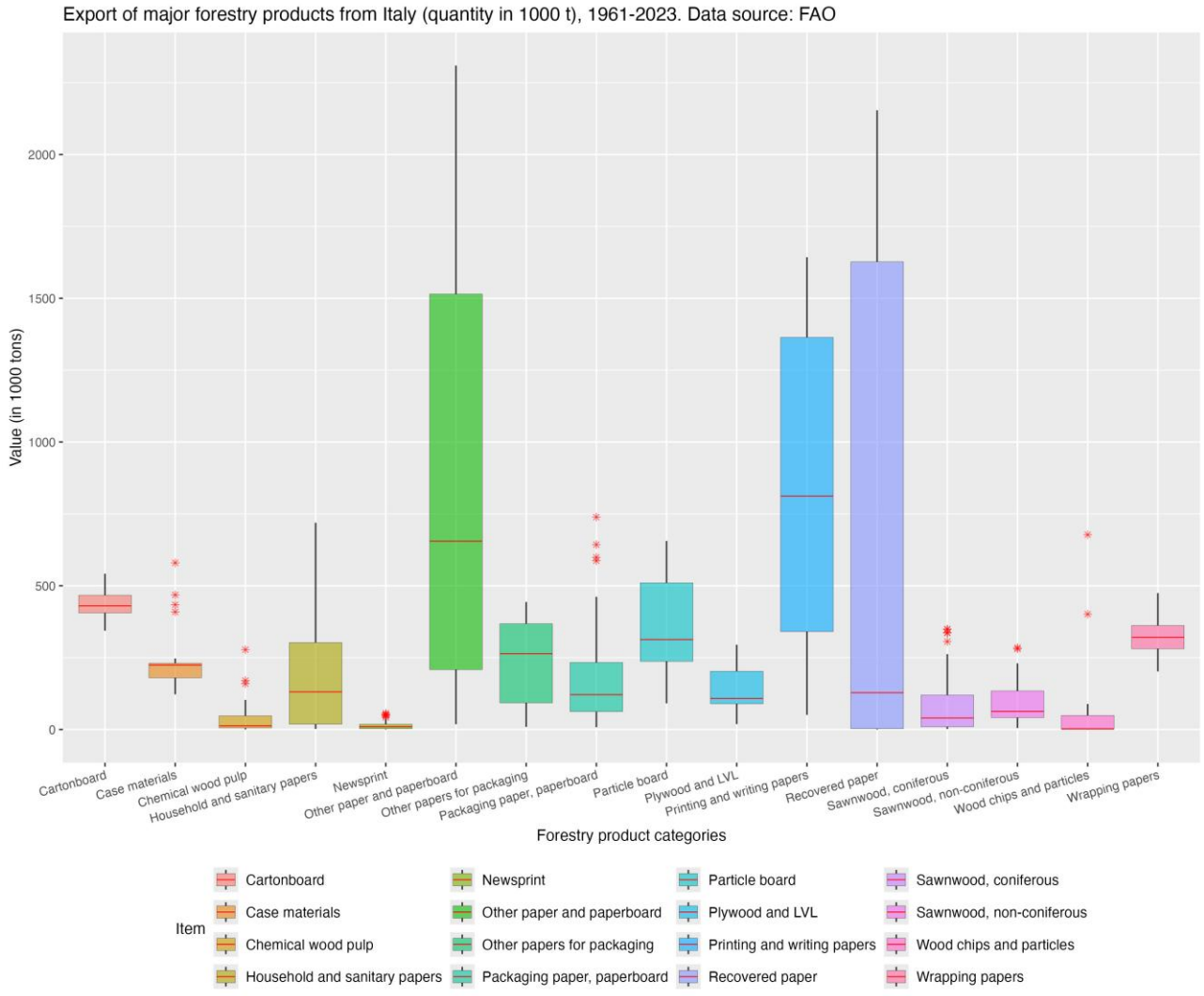

Fig. 5. Forestry products export quantity in Italy (in t), 1961-2023. Data source: FAO (category: "Forestry production and trade"). Software: R. Graph source: made by the author.

Hence, the exported of newsprint paper increased from 9000 in 1961 to 354.396 in 2023. Besides, the recovered pulp production as a residual volume increased significantly from 3754 until 2154.058.

The dynamics of the recovered pulp production was calculated and compared for recent decades for cartonboard (dropped from 401,471 to 343,670 tons), case materials (148,000 increased to 468,151 tons) and wood pulp (800 increased to 159284 tons). The production of cartonboard is obviously decreased comparing to the production of packaging paper and paperboard which demonstrated more than 739000 thousand tons recently. Such dynamics in pulp and paper export production is shaped by a strategic focus on decarbonization and a strong EU market position. Key dynamics include a push for greater energy efficiency, electrification, and low-carbon fuels to meet climate goals. The market also faces challenges from high energy costs and digital substitution, while opportunities exist in high-quality, sustainable packaging demand driven by luxury and food sectors.

Table 1 shows the calculated and summarized trends between diverse land cover types of Italy during recent decades, based on MODIS data and FAO repository.

Table. 1. Forestry export value in Italy: dynamics for 2003-2023 (in millions USD). Data source: FAO (category: “Forestry production and trade”).

Year	Case materials	Household and sanitary papers	Newsprint	Paper and paperboard	Recovered paper	Wrapping and packaging paper
2003	73,8	185,71	2,8	1152,5	65,2	371,1
2004	99,9	201,75	6,9	1297,6	71,7	39,1
2005	44,91	238,93	9,16	1242,3	82,54	491,08
2006	108,95	1062,7	9,04	2327,03	102,6	506,1
2007	121,52	1219,81	5,74	2607,97	178,5	498,35
2008	101,09	1253,86	8,51	2696,69	259,61	535,43
2009	46,5	1133,53	8,36	2277,48	207,51	434,74
2010	140,91	374,68	3,89	1619,83	319,18	478,25
2011	175,68	415,83	2,23	1698,99	396,31	404,12
2012	164,45	379,02	2,1	1716,77	336,52	524,63
2013	183,72	431,31	1,47	1943,56	297,96	568,04
2014	164,41	458,51	0,82	2029,3	279,14	598,33
2015	164,9	389,86	0,79	1798,48	264,8	515,74
2016	165,03	350,65	1,51	1751,2	302,33	517,57
2017	177,4	361,75	1,11	2011,86	349,86	570,77
2018	185,32	473,24	1,3	2109,32	309,69	581,22
2019	159,59	413,88	0,78	1843,38	205,44	464,02
2020	224,01	469,96	1,2	1875,84	201,34	402,66
2021	413,81	482,39	3,12	2375,17	321,83	481,99
2022	366,51	602,04	3,38	2603,35	298,21	567,57
2023	274,25	516,56	1,42	2115,07	271,09	482,04

The largest values for the imported products in Italy were found for the following seven categories (**Fig. 6.**): 1) Sawnwood, coniferous; 2) Chemical wood pulp; 3) Other paper and paperboard; 4) Chemical wood pulp, sulphate, bleached; 5) Industrial roundwood, coniferous (export/import); 6) Sawlogs and veneer logs, non-coniferous; 7) Printing and writing papers.

Italy relies heavily on imports for its coniferous sawn wood production, with a significant portion of its demand met by foreign sources. While domestic production has seen increases, it is insufficient to meet consumption, which is estimated to be around 6-7 M m^3 annually, with import accounting for ca. 90%. Major suppliers are primarily within the EU and other regions. The graph in **figure 6** shows that the peak in the import of the coniferous sawnwood was on 2007 when it reached 6438000 m^3 imported to the country. Sawlogs and veneer logs are essential raw materials imported to Italy, as essential items for production of sawn timber for construction, furniture, and pallet. Since Italy is a major player in the EU furniture production, recognized globally for high-end and design-focused pieces and a top exporter, it has a high dependance on the sawlogs and veneer logs. Fig. 6 shows the peaks in the volume of sawlogs and veneer logs imported to Italy, reached 1491600 m3 in 1980.

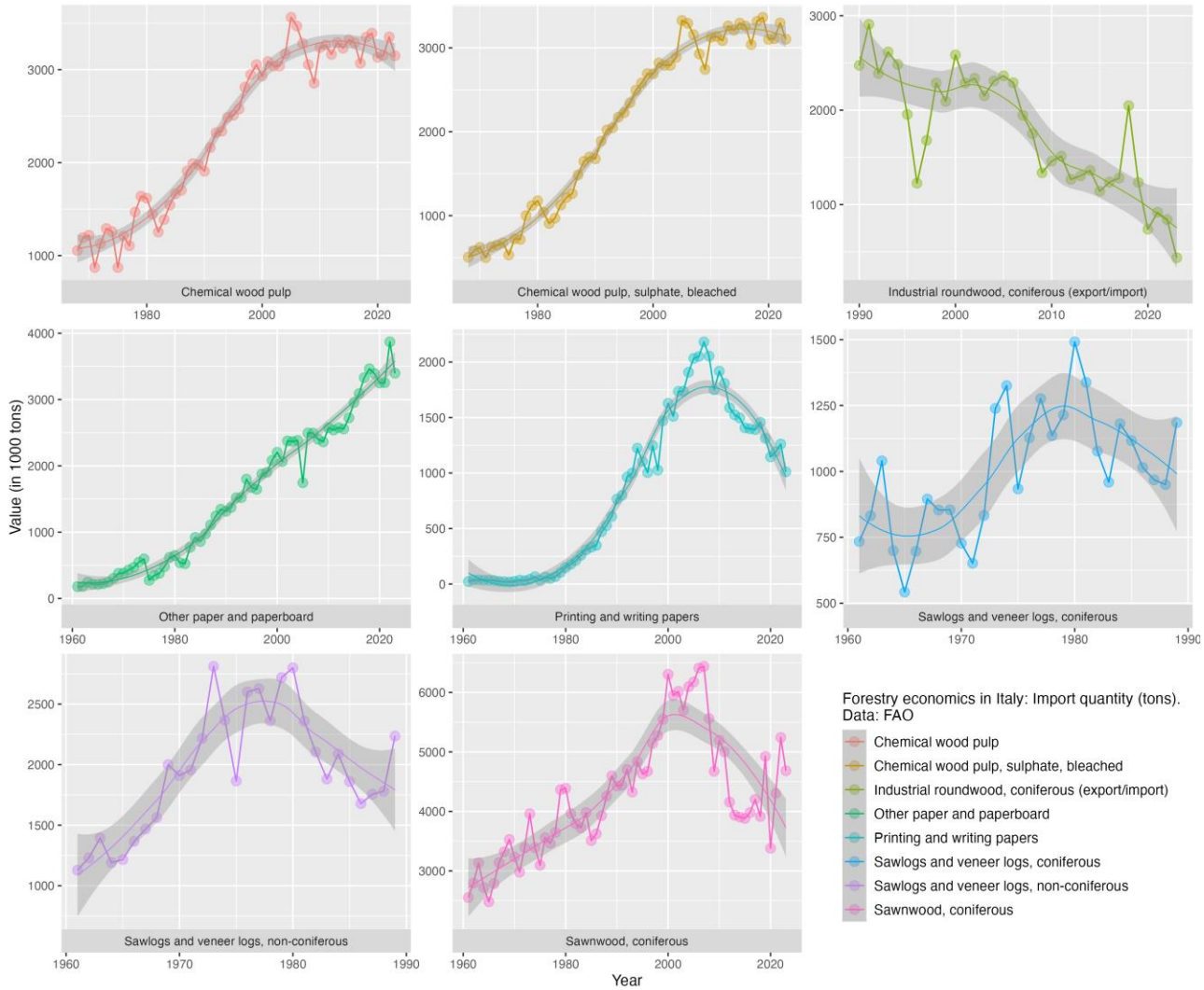

Fig. 6. Forestry import quantity (tons). Data source: FAO (category: "Forestry production and trade"). Software: R. Graph source: made by the author.

Italy imports significant quantities of chemical pulp for paper production, with key supplying countries including France, Germany, and the US. A major supplier of chemical pulp for fibrous cellulosic material, is France. The highest volume of the imported chemical pulp is reached in 2019 with 3392219 m^3. Due to the high demand for the industrial roundwood, Italy remains a significant net importer of this forestry product. While the country has a substantial forest cover, it imports roundwood additionally to meet the high demand of its industries, especially furniture. In 2022, the average import price was ca. $96 per m^3, with a 14% increase from previous years (**Fig. 6.**).

The analysis is focused on forestry sector to compare economic flows in wooden and pulp-and-paper production material in Italy(import/export) with environmental situation on forest stands. The analyses revealed balanced development in forestry industry and forest protection. An increase of forest areas over the Apennines Peninsula was detected, along with the decrease in pulp-and-paper production which demonstrated a clear shift towards the growth of e-commerce and distribution of e-books. Data-driven integration of FAO data, statistical analysis and methods of R statistical software present the reliable source of information for the analysis of the relationship between economic activity and its environmental impact in forest industry. Monitoring of the economic dynamics was performed with regards to the environmental impacts.

The analysis of the dynamics in timber and pulp-and-paper industry of Italy is characterized by a push for efficiency, sustainability, and diversification. The concept of the economic-driven environmental protection consists in the importance of nature conservation for society and climate, maintaining sustainable development. The FAO data on category “Forestry production and trade” was analyzed for important categories of forestry import in Italy for recent two decades (**Table 2**).

The presented data on forestry sector development of Italy and dynamics of land cover types with the role of forest and natural reserves is intended for analysis of sustainable economic growth. Traditionally, the economic growth is linked to environmental pressure through increased resource use. Nevertheless, it also leads to the environmental improvement through innovations in green technology and the development of environmental goods and services. This complex relationship in forestry industry is a focus of environmental economics aimed at sustainable development. The statistical findings support and policy recommendation integrates climate adaptation for sustainable development goals: new directives in Italy (National Climate Change Adaptation Plan (PNACC), 2023), which provides guidance for integrating climate adaptation into planning procedures on environmental protection, and updated the guidelines for adaptation strategies and plans in silviculture and forestry.

The national directives for the wood industry in Italy include compliance with the European Timber Regulation (EUTR) through national legislation like Legislative Decree n. 178/2014. Key directives involve sustainable forest management, supported by initiatives like the revised Italian Forest Stewardship Standard, and policies promoting the management and use of domestic wood resources. These directions are in line with the presented results which demonstrate major trends in forestry sector of the country. To summarize the key results based on the FAO data analysis, the main issues in forestry industry of Italy, include the following items:

- The highest land cover type in Italy is occupied by forests following by crops, according to the MODIS data, which proves favorable situation in environmental sense (**Fig. 2.**, left).
- Increased emissions from forest during 20th century, according to the FAO data, which include relevant ecosystem carbon pools, according to FAO data: aboveground biomass, below ground biomass, dead wood, litter, soil organic carbon, and greenhouse gases (CO_2, CH_4, N_2O). The emissions were increasing during 20th century, but now the situation stabilized due to the undertaken measures of environmental protection (**Fig. 2.**, right).
- Expanding forest area due to rural abandonment (**Fig. 3.**)
- Growing focus on digitalization and paper production (**Fig. 4.**)
- Dynamic in commerce with high values in export of paper products – packaging paper, recovered paper, printing and writing paper (**Fig. 5.**)
- shift in forest management towards biodiversity and resilience with a high dependence on imported wood despite increased domestic production (**Fig. 6.**).

Table 2. Forestry import value in Italy in two decades (in millions USD) by categories. Data source: FAO.

Year	Chemical wood pulp	Chemical wood pulp, sulphate, bleached	Industrial round-wood, coniferous	Other paper and paperboard	Printing and writing papers	Wood charcoal	Wood residues	Sawn-wood, coniferous
2003	1616,93	1483,82	182,31	1757,87	1490,75	12,85	40,52	1074,99
2004	1735,39	1576,61	202,78	1866,08	1695,77	15,7	73,82	1253,25
2005	1813,69	1691,92	185,3	1191,69	1761,88	18,4	72,76	1214,03
2006	1974,44	1870,76	203,08	2030,04	1819,06	19,96	100,89	1462,77
2007	2261,49	2166,84	211,69	2299,88	2143,98	23,5	49,59	1672,18
2008	2310,7	2216,05	205,09	2404,84	2192,37	26,73	95,56	1427,28
2009	1587,88	1526,54	143,91	2005,16	1753,9	28,65	106,48	1075,6
2010	2390,14	2312,72	153,99	2192,03	1815,34	25,58	257,44	1225,54
2011	2388,8	2300,29	172,2	2509,08	1892,64	28,65	336,62	1306,76
2012	1991,42	1944,35	125,53	2255,65	1526,87	26,93	36,28	981,13
2013	2160,23	2138,4	133,14	2531,39	1484,14	27,82	42,31	966,42
2014	2090,01	2072,66	141,93	2624,55	1446,92	28,5	42,75	989,13
2015	2098,21	2080,03	102,96	2314,03	1133,19	25,72	14,16	818,24
2016	1823,9	1804,12	108,02	2311,96	1133,14	26,54	12,62	813,41
2017	1981,1	1960,33	119,69	2814,68	1161,18	24,01	16,31	892,71
2018	2526,46	2500,82	137,35	3119,09	1330,14	27,02	19,22	940,22
2019	2138,5	2116,03	82,79	2677,55	1168,6	27,16	17,1	801,68
2020	1576,71	1553,55	53,39	2419,56	944,1	30,31	15,99	733,4
2021	2115,45	2081,1	85,25	2982,72	1053,43	32,93	25,74	1464,82
2022	2704,36	2656,01	85,63	4071,75	1438,67	33,56	23,87	1553,82
2023	2221,86	2182,56	66,79	3258,93	1226,39	41,04	28,88	1116,79

Hence, this work highlighted the environmental perspectives in Italy and dynamics of forests with favorable situation. The rate of forest expanding is ca. 100,000 ha/year, i.e., 30% of land recently dedicated to biodiversity conservation. At the same time, the economics of the country experience restructuring and shift towards less printing and more e-based productions, which can be seen on the demonstrated graphs with dynamics of import and export products in forestry sector.

The major trend is driven by rising demand for e-commerce, e-publishing and shifts towards packaging and online commercialization of the news (decline of volume of paper-based books and printed newspapers). Hence, the products that naturally used high consumption in forestry industry now decreased the demand for pulp-and-paper products. Such trends also face pressure to reduce its high energy and water consumption. Modelling and visualization of FAO datasets highlighted such dynamics in pulp-and-paper industry through time series analysis of categories analyzed by variables. Besides the environmental applications, updated datasets on forestry industry can be used for monitoring economic growth to reveal the characteristics of market and their correlation with the use of natural resources, e.g., forest cuts and logging. This enables to detect cases of overuse of resources as information for decision taking on land protection.

4. Discussion

Highlighting and interpreting the links between economic trends in forestry and environmental dynamics of land cover types requires integration of diverse large datasets (Pudzis et al., 2025; Vallejos et al., 2025; Lu et al., 2025). Interpreting such data enables to reveal and detect precious information with a particular focus on forest areas. Such evaluation of landscape dynamics is possible using data analysis for environmental monitoring. For instance, climate-related hazards (rise in temperature, decline in precipitation) can be evaluated using time series (Barlık, 2025; Yi et al., 2025; Lemenkova 2021). However, the use of such information relies on the effective methods of data processing. This becomes especially challenging in the era of big data and enables to use additional methods of data analysis (Frantz et al., 2022; Li & Dong, 2022). The solutions to this problem are provided by advanced statistical tools which present accurate and standardized methods to automate the statistical workflow (Malviya et al., 2016; Salloum et al., 2019). Processing multi-format statistical and spatial data for environmental monitoring and economic analysis is possible using R, which enables automated data analysis through the advanced algorithms embedded in its libraries.

In this study, we demonstrated the use of R for highlighting the inks between the environmental and economic sectors of forestry in Italy. The dynamics of the forestry sector of Italy recently undergone a restructuring. The data from FAO reflect a development forestry sector driven by digital transformation of business structure, increasing forest area due to land abandonment, and the need for new management strategies. This includes a shift toward sustainable practices, increase in nature protection actions, importance of non-timber products (firewood), and challenges related to forest fires and adaptive measures to climate change in Italy following the EU regulations.

The development of statistical instruments, such as R, provided environmentalists and economists with a principally new approach to data processing through scripts. This enables to rapidly process large volumes of data, such as FAO repositories, and extract information to find correlations between the variables. Statistical data are also well suitable for

integrated monitoring that includes multi-disciplinary domains, such as environmental economics in forestry.

With rapid development of programming, data analysis and modelling became more accurate and systematic due to the diverse and constantly developing statistical techniques. Nowadays, dynamic monitoring in large repositories containing economic and climate data become possible using such tools as R. Advanced statistical tools makes data analysis systematic and precise. Libraries of R enable to evaluate the distribution of diverse variables across various countries in different time periods which shifts research into the novel perspectives of big data analysis:

1. The economics of forestry in Italy reflects industrial demand for forest products. Therefore, the import and export of forestry products include lumber, paper, and biofuels, as well as non-market ecosystem services. The dynamics of forestry sector is driven by social factors and policy on market profitability and sustainability.
2. Forest areas are influenced by climate change through the effects of temperature on soil, hydrology and vegetation, rainfall patterns, and variations in weather which control the variability of natural habitats and wildlife. Climate change also drives the dynamics of land cover types through reforestation, and cause habitat changes.
3. Forestry industry depends on a renewable forest resource. At the same time, it relies on sustainable management practices that balance harvesting with regeneration of forest stands. Key factors of forestry sector include ecosystem integrity, effective governance, and economic drivers. The latter include market demand for wood and paper products, and technological innovation in processing and practices.

Monitoring forest industry in the context of economics and environmental sustainability is particularly actual for land management. Specifically for Italy, understanding structure of forestry sector and dynamics in this industry is especially difficult as it includes the balance between the economic demands and protection of natural resources.

Conclusions

Forest management in Italy recently undergone significant changes and restructuring. It is aimed at sustainable use and protection create novel opportunities in forestry sector which focus on gradual improving of management scheme, expanding tree plantations and forest stands. Nowadays, the country undertakes measures on optimization of the resource use and strengthening the contribution of the forestry sector to rural development. This is especially valuable for southern regions of the country which have dominantly rural profile. The National Forestry Strategy of the country aims to promote sustainable production and consumption of forest products and woods, and has allocated additional resources for forestry infrastructure. The formation of trademarks and development of different products depend on internal and international market (mostly, EU-based) and processes related to industrial improvements in the forestry sector. The actuality of monitoring statistical data in forestry industry has significantly increased in Italy along with reforestation and increased need for forestry products in the EU market.

This study employed the advanced tools of R for statistical analysis of forest production and trade in Italy and identification of variations in regional scale. Using multi-source FAO-based data, different environmental and economic characteristics of forestry were analyzed for diverse parameters. This enabled to analyze major trends in the economic and

environmental development in contrasting sectors of forestry across Italy (paper and pulp production, import and export, revenues, volume of products). Economic trends in forest industry is driven by a complex combination of financial and environmental factors that include direct revenue from timber and wood products, the value of ecosystem services, and climate finance. Italy is actively restructuring its forestry management to adapt to climate change. This includes strong measures on promoting climate-resilient silviculture: planting mixed stands of conifers and broadleaves to increase resilience to extreme climate events in northern Italy, enriching forest stands with local, native species, adapted to climate setting and practicing forest regeneration for carbon sequestration. Such activities strengthen the environmental function of forest stands on carbon sequestration and climate regulation. Regenerating new forest stands in central Italy has positive impacts on biodiversity, soil stability, and water cycles. Measures on restoring degraded areas enable ecological reforestation of lands degraded by erosion or fire in southern Italy.

Balancing economic value of forests and their role in nature protection can be complex. Finding optimal balance between societal development through monetization of forest products (wood, timber, pulp and paper, etc.) and protecting natural parks and reserves involve both risks and opportunities. For example, natural factors of forest stands can be primarily categorized as climate-related issues (patterns of precipitation and variations in temperature) and managing climate change impacts to capitalizing on carbon credits. Therefore, statistical data analysis to support studies focused on the analysis of forest role in economics and environment are essential for land management and decision making. This paper contributed to such issues through R-based statistical analysis of FAO dataset for analysis of trends in forestry of Italy.

The values of forest in ecological balance can not be overestimated. The processes of climate and environment affect forest landscapes and form ecosystems that reflect a complex interplay of factors: climate, topography, soil, hydrologic processes, and anthropogenic activities. Analysis of forest health enables to accurately evaluate the impacts of the processes and the effects affecting landscape structure, associated with climate change (Slepetiene et al., 2025; Chen et al., 2025; Lemenkova, 2025c; Shi et al., 2025). The long-term perspectives and benefits of forest studies consist in the applications for theoretical statistical approach which can be of interest for environmental analysis and land planners (applied ecology and landscapes in forest areas).

Future studies on forest management can employ the integrated methods of statistical analysis, GIS and RS to monitor forestry in economic context. Using datasets can present new insights into the economic-environmental analysis of the forestry industry to better understand their resilience and economic value. The benefits of the data analysis of forest landscapes and monitoring their economic role for finding balance between the society and environment.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Adams MA & Attiwill PM (1984). Patterns of nitrogen mineralisation in 23-year old pine forest following nitrogen fertilising.

Forest Ecology and Management, 7(4): 241–248. https://doi.org/10.1016/0378-1127(84)90001-X
2. Adger WN, Brown K, Cervigni R, & Moran D (1995) Total Economic Value of Forests in Mexico. Ambio 24(5): 286–296. http://www.jstor.org/stable/4314349
3. Adun H, Bamisile O, Mukhtar M, Dagbasi M, Kavaz D & Oluwasanmi A (2021) Novel Python-based “all-regressor model” application for photovoltaic plant-specific yield estimation and systematic analysis. Energy Sources, Part A: Recovery, Utilization, and Environmental Effects, 47(1), 7480–7498. https://doi.org/10.1080/15567036.2021.1921886
4. Agrawal A, Chhatre A & Hardin R (2008). Changing governance of the world’s forests. Science 320(5882):1460–62. https://doi.org/10.1126/science.1155369
5. Anderson E, Chlumsky R, McCaffrey D, Trubilowicz J, Shook KR & Whitfield PH (2018). R-functions for Canadian hydrologists: a Canada-wide collaboration. Canadian Water Resources Journal / Revue Canadienne Des Ressources Hydriques, 44(1): 108–112. https://doi.org/10.1080/07011784.2018.1492884
6. Arts B & Buizer M (2009) Forests, discourses, institutions. A discursive-institutional analysis of global forest governance. Forest policy and economics 11 (5–6): 340–347. https://doi.org/10.1016/j.forpol.2008.10.004
7. Barlık, N. (2025). Long-Term Temporal Analysis of Climate Variables for Erzurum. Atmosphere, 16(11), 1250. https://doi.org/10.3390/atmos16111250
8. Barnes WL, Pagano TS, & Salomonson VV (1998) Prelaunch characteristics of the Moderate Resolution Imaging Spectroradiometer (MODIS) on EOS-AM1, in IEEE Transactions on Geoscience and Remote Sensing 36 (4): 1088-1100. https://doi.org/10.1109/36.700993
9. Bilina R, & Lawford S (2012). Python for Unified Research in Econometrics and Statistics. Econometric Reviews, 31(5): 558–591. https://doi.org/10.1080/07474938.2011.553573
10. Bockstael NE, Freeman AM, Kopp RJ, Portney PR, Smith VK (2000) On measuring economic values for nature. Environmental Science & Technology 34 (8): 1384–1389. https://doi.org/10.1021/es9906731
11. Borghesi S, Cainelli G, Mazzanti M (2015) Linking emission trading to environmental innovation: evidence from the Italian manufacturing industry. Research Policy, 44(3):669–683. https://doi.org/10.1016/j.respol.2014.10.014
12. Boyd J & Banzhaf S (2007) What Are Ecosystem Services? The Need for Standardized Environmental Accounting Units. Ecological Economics 63: 616–626. https://doi.org/10.1016/j.ecolecon.2007.01.002
13. Burnett M & Davis C (2002). Getting out the cut. Politics and national forest timber harvests, 1960–1995. Administration & Society, 34 (2): 202–228. https://doi.org/10.1177/0095399702034002004
14. Chaves MED, Soares LGD, Barros GHV, Pessoa ALF, Elias RO, Golzio AC, Conceição K V, & Morais FJO (2025). Mixing Data Cube Architecture and Geo-Object-Oriented Time Series Segmentation for Mapping Heterogeneous Landscapes. AgriEngineering, 7(1): 19. https://doi.org/10.3390/agriengineering7010019

15. Chen LP (2021). Practical Statistics for Data Scientists: 50+ Essential Concepts Using R and Python: by Peter Bruce, Andrew Bruce, and Peter Gedeck, O'Reilly Media Inc., Boston, United States 2020. 368 pp. Technometrics, 63(2), 272–273. https://doi.org/10.1080/00401706.2021.1904738
16. Chen Y, Zhang R, Dehghanifarsani L & Amani-Beni M (2025). Dynamics and Drivers of Ecosystem Service Values in the Qionglai–Daxiangling Region of China's Giant Panda National Park (1990–2020). Systems, 13(9), 807. https://doi.org/10.3390/systems13090807
17. Choi YJ & Asilkalkan A (2019) R Packages for Item Response Theory Analysis: Descriptions and Features. Measurement: Interdisciplinary Research and Perspectives, 17(3), 168–175. https://doi.org/10.1080/15366367.2019.1586404
18. Cribari-Neto F & Zarkos SG (1999). R: yet another econometric programming environment. Journal of Applied Economics, 14: 319-329. https://doi.org/10.1002/(SICI)1099-1255(199905/06)14:3<319::AID-JAE533>3.0.CO;2-Q
19. Fan, J., Yao, Y., Li, Y., Liu, L., Xie, Z., Zhang, X., Kan, Y., Zhang, L., Qiu, F., Qu, J., & Shi, D. (2025). A Simplified Sigmoid-RH Model for Evapotranspiration Estimation Across Mainland China from 2001 to 2018. Forests, 16(7), 1157. https://doi.org/10.3390/f16071157
20. Farnworth EG, Tidrick TH, Smathers WM & Jordan CF (1983). A synthesis of ecological and economic theory toward more complete valuation of tropical moist forests. International Journal of Environmental Studies, 21(1): 11–28. https://doi.org/10.1080/00207238308710059
21. Frantz, D., Hostert, P., Rufin, P., Ernst, S., Röder, A., & van der Linden, S. (2022). Revisiting the Past: Replicability of a Historic Long-Term Vegetation Dynamics Assessment in the Era of Big Data Analytics. Remote Sensing, 14(3), 597. https://doi.org/10.3390/rs14030597
22. Gaston L, Blazier M, Beasley J, Dodla S, Felicien W & Clason T (2019). Silvopasture Switchgrass Fertilized with Poultry Litter: Nutrient Removal, Soil Fertility, and Runoff Water Quality. Communications in Soil Science and Plant Analysis, 50(8): 948–958. https://doi.org/10.1080/00103624.2019.1594878
23. Goldstein RM, Wang L, Simon TP & Stewart PM (2002) Development of a Stream Habitat Index for the Northern Lakes and Forests Ecoregion. North American Journal of Fisheries Management, 22(2): 452–464. https://doi.org/10.1577/1548-8675(2002)022<0452:DOASHI>2.0.CO;2
24. Hu, G., Wang, T., Wan, M., Bao, W., & Zeng, W. (2022). UAV remote sensing monitoring of pine forest diseases based on improved Mask R-CNN. International Journal of Remote Sensing, 43(4), 1274–1305. https://doi.org/10.1080/01431161.2022.2032455
25. Imaya A, Ohta S, Tanaka N & Inagaki Y (2005) General Chemical Properties of Brown Forest Soils Developed from Different Parent Materials in the Submontane Zone of the Kanto and Chubu Districts, Japan. Soil Science and Plant Nutrition, 51(6): 873–884. https://doi.org/10.1111/j.1747-0765.2005.tb00122.x
26. Jindal R, Kunwar AK, Kaur A & Jakhar BS. (2020). Predicting the Dynamics of Forest Fire Spread from Satellite Imaging

Using Deep Learning. 2020 International Conference on Electronics and Sustainable Communication Systems (ICESC), Coimbatore, India, 344-350, https://doi.org/10.1109/ICESC48915.2020.9155734

27. Joyo FH, Nastasi B, Astiaso D (2025) Garcia Decarbonization pathways for the pulp and paper industry: a comprehensive review. Renewable and Sustainable Energy Reviews, 223: 116070, https://doi.org/10.1016/j.rser.2025.116070
28. Junttila S, Vastaranta M, Hämäläinen J, Latva-käyrä P, Holopainen M, Hernández Clemente R, Hyyppä H, Navarro-Cerrillo RM (2016). Effect of forest structure and health on the relative surface temperature captured by airborne thermal imagery – Case study in Norway Spruce-dominated stands in Southern Finland. Scandinavian Journal of Forest Research, 32(2): 154–165. https://doi.org/10.1080/02827581.2016.1207800
29. Justice CO, Vermote EF, Townshend JR, DeFries RS, Roy DP, Hall DK, Salomonson VV, Privette JL, Riggs GA, Strahler AH, Lucht W, Myneni RB, Knyazikhin Y, Running SW, Nemani RR, Wan Z, Huete AR, Leeuwen WV, Wolfe RE, Giglio L, Muller J, Lewis P, & Barnsley MJ (1998). The Moderate Resolution Imaging Spectroradiometer (MODIS): land remote sensing for global change research. IEEE Transactions on Geoscience and Remote Sensing, 36, 1228–1249. https://doi.org/10.1109/36.701075
30. Kahn RA & Sayer AM (2023). Satellites and satellite remote sensing $|$ aerosol measurements. Reference Module in Earth Systems and Environmental Sciences. Elsevier. https://doi.org/10.1016/B978-0-323-96026-7.00001-1
31. Keleş S (2018). An assessment of hydrological functions of forest ecosystems to support sustainable forest management. Journal of Sustainable Forestry, 38(4): 305–326. https://doi.org/10.1080/10549811.2018.1547879
32. Kesgin Atak B, & Tonyaloğlu EE (2025). Assessing the Spatiotemporal Dynamics of Regional Ecosystem Health in Aydın Province, Türkiye. Sustainability, 17(23), 10522. https://doi.org/10.3390/su172310522
33. Klaučo M, Gregorová B, Stankov U, Marković V, Lemenkova P (2013) Determination of ecological significance based on geostatistical assessment: A case study from the Slovak Natura 2000 protected area. Open Geosciences, 5(1): 28-42. https://doi.org/10.2478/s13533-012-0120-0
34. Klaučo M, Gregorová B, Koleda P, Stankov U, Marković V, Lemenkova P (2017) Land planning as a support for sustainable development based on tourism: A case study of Slovak rural region. Environmental Engineering and Management Journal, 16(2):449-458. https://doi.org/10.30638/eemj.2017.045
35. Kleiber C & Zeileis A (2008) Applied econometrics with R. Springer Science & Business Media. https://doi.org/10.1007/978-0-387-77318-6
36. Knauf M (2015) An analysis of wood market balance modeling in Germany. Forest Policy and Economics, 50:319–326. https://doi.org/10.1016/j.forpol.2014.09.013
37. Kocaman B, & Ağaçcıoğlu H (2025). Assessment of the Impact of Land Use/Land Cover Changes on Carbon Emissions Using Remote Sensing and Deep Learning: A Case Study of the Kağıthane Basin. Sustainability, 17(23), 10690. https://doi.org/10.3390/su172310690

38. Lemenkova P (2019a). Testing Linear Regressions by StatsModel Library of Python for Oceanological Data Interpretation. Aquatic Sciences and Engineering, 34(2): 51-60. https://doi.org/10.26650/ASE2019547010
39. Lemenkova P (2019b). Statistical Analysis of the Mariana Trench Geomorphology Using R Programming Language. Geodesy and Cartography, 45(2): 57-84. https://doi.org/10.3846/gac.2019.3785
40. Lemenkova P (2021) Evaluating the Performance of Palmer Drought Severity Index (PDSI) in Various Vegetation Regions of the Ethiopian Highlands. Acta Biologica Marisiensis 4(2):14–31. https://doi.org/10.2478/abmj-2021-0010
41. Lemenkova, P. (2022a). Mapping Climate Parameters over the Territory of Botswana Using GMT and Gridded Surface Data from TerraClimate. ISPRS International Journal of Geo-Information, 11(9): 473. https://doi.org/10.3390/ijgi11090473
42. Lemenkova, P. (2022b). Handling Dataset with Geophysical and Geological Variables on the Bolivian Andes by the GMT Scripts. Data, 7(6):74. https://doi.org/10.3390/data7060074
43. Lemenkova P (2024a) Deep Learning Methods of Satellite Image Processing for Monitoring of Flood Dynamics in the Ganges Delta, Bangladesh. Water, 16 (8):1141 https://doi.org/10.3390/w16081141
44. Lemenkova P (2024b). Exploitation d'images satellitaires Landsat de la région du Cap (Afrique du Sud) pour le calcul et la cartographie d'indices de végétation à l'aide du logiciel GRASS GIS. Physio-Géo, 20(1): 113-129. https://doi.org/10.4000/11pyj
45. Lemenkova P (2025a). On forest hydrology in South Tyrol: Processes, modelling and assessment. Agriculture and Forestry, 71(2): 7-22. https://doi.org/10.5281/zenodo.15785388
46. Lemenkova P (2025b) Automation of image processing through ML algorithms of GRASS GIS using embedded Scikit-Learn library of Python. Examples and Counterexamples 7:100180. https://doi.org/10.1016/j.exco.2025.100180
47. Lemenkova P (2025c) Machine Learning Algorithms of Remote Sensing Data Processing for Mapping Changes in Land Cover Types over Central Apennines, Italy. Journal of Imaging, 11(5):153. https://doi.org/10.3390/jimaging11050153
48. Li, Z., & Dong, J. (2022). Big Geospatial Data and Data-Driven Methods for Urban Dengue Risk Forecasting: A Review. Remote Sensing, 14(19), 5052. https://doi.org/10.3390/rs14195052
49. Li X, Nour MH, Smith DW & Prepas EE (2010). Neural networks modelling of nitrogen export: model development and application to unmonitored boreal forest watersheds. Environmental Technology, 31(5): 495–510. https://doi.org/10.1080/09593330903527880
50. Lin W, Chen J, Zheng Y, Dai Y (2019) Effects of the eu emission trading scheme on the international competitiveness of pulp-and-paper industry. Forest Policy and Economics, 109:102021. https://doi.org/10.1016/j.forpol.2019.102021
51. Lombardo E (2022) An overview of bamboo cultivation in southern Italy. Advances in Bamboo Science, 1:100002. https://doi.org/10.1016/j.bamboo.2022.100002
52. Lu, S., Lu, H., Zhang, C., Miao, C., & Kizos, T. (2025). Quantitative Impacts of Socio-Economic Changes on REDD+

Benefits in Xishuangbanna Rainforests. Forests, 16(1), 120. https://doi.org/10.3390/f16010120
53. Luo J, Wu X, Gao Y, Cai Y, Yang L, Xiong Y, Yang Q, Liu J, Li Y, Deng Z, Wang Q, Li B (2025) Spatiotemporal Variations and Seasonal Climatic Driving Factors of Stable Vegetation Phenology Across China over the Past Two Decades. Remote Sensing, 17(20):3467. https://doi.org/10.3390/rs17203467
54. Malviya A, Udhani A & Soni S (2016) R-tool: Data analytic framework for big data. Symposium on Colossal Data Analysis and Networking (CDAN), Indore, India, 1-5, doi: https://doi.org/110.1109/CDAN.2016.7570960
55. National Climate Change Adaptation Plan (PNACC), 2023. URL: https://www.isprambiente.gov.it/en/news/the-national-plan-for-adaptation-to-climate-change-pnacc-approved (accessed: 20/11/2025).
56. Peabody MR (2023). Comparison of R Packages for Automated Test Assembly with Mixed-Integer Linear Programming. Measurement: Interdisciplinary Research and Perspectives, 21(1): 55–61. https://doi.org/10.1080/15366367.2022.2151081
57. Pudzis, E., Kundziņa, A., & Druķis, P. (2025). The Role and Potential of Timber in Construction for Achieving Climate Neutrality Objectives in Latvia. Sustainability, 17(18), 8247. https://doi.org/10.3390/su17188247
58. Raivio S, Normark E, Pettersson B & Salpakivi-Salomaa P (2001) Science and the Management of Boreal Forest Biodiversity – Forest Industries' Views. Scandinavian Journal of Forest Research, 16(sup003), 99–104. https://doi.org/10.1080/02827580130009070 8
59. Ruhl PJ, Kellner KF & Dunning JB Jr. (2020) Ecological factors explain habitat associations of mature-forest songbirds in regenerating forest clearcuts. The Wilson Journal of Ornithology, 132(1), 145–158. https://doi.org/10.1676/1559-4491-132.1.145
60. Salomonson, V.V., Barnes, W., Masuoka, E.J. (2006). Introduction to MODIS and an Overview of Associated Activities. In: Qu, J.J., Gao, W., Kafatos, M., Murphy, R.E., Salomonson, V.V. (eds) Earth Science Satellite Remote Sensing. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-540-37293-6_2
61. Salloum S, Huang JZ & He Y. (2019) Random Sample Partition: A Distributed Data Model for Big Data Analysis. IEEE Transactions on Industrial Informatics, 15(11), 5846-5854, Nov. 2019, https://doi.org/10.1109/TII.2019.2912723
62. Shahare G, Manchalwar J, Wankhede K, Meshram S, Kalbande K & Wyawahare N (2024) Exploring Multidisciplinary Data Visualization and Data Analysis with R. 2nd International Conference on Advancements and Key Challenges in Green Energy and Computing (AKGEC), Ghaziabad, India, 1-6. https://doi.org/10.1109/AKGEC62572.2024.10869196
63. Shi, L., Zhang, X., & Halik, Ü. (2025). The Driving Mechanism and Spatio-Temporal Nonstationarity of Oasis Urban Green Landscape Pattern Changes in Urumqi. Remote Sensing, 17(17), 3123. https://doi.org/10.3390/rs17173123
64. Squire RO, Flinn DW & Campbell RG (1991). Silvicultural research for sustained wood production and biosphere conservation in the pine plantations and native eucalypt forests of south-eastern

Australia. Australian Forestry, 54(3), 120–133. https://doi.org/10.1080/00049158.1991.10674568

65. Shojaie shami A, Yarali N, Gholami DM & Adeli K (2021). Valuation of ecosystem functions and services: Dehdez forest in Iran. Journal of Forest Research, 26(5), 358–366. https://doi.org/10.1080/13416979.2021.1939921
66. Skulska I, Colaço MC, Aggarwal S, Didier H, Monteiro M do L & Rego FC (2019). Assessment of Portuguese Community Forestry using the Voluntary Guidelines on the Responsible Governance of Tenure and FAO Community-Based Forestry Framework. Society & Natural Resources, 33(1), 101–121. https://doi.org/10.1080/08941920.2019.1660934
67. Slepetiene, A., Belova, O., Fastovetska, K., Dinca, L., & Murariu, G. (2025). Managing Boreal Birch Forests for Climate Change Mitigation. Land, 14(9), 1909. https://doi.org/10.3390/land14091909
68. Sona DR, Greeshma A, Reddy GSB & Thomas SB (2025) Hybrid CNN-Ensemble Framework for Robust Floral Species Recognition. 2025 International Conference on Sensors and Related Networks (SENNET) Special Focus on Digital Healthcare(64220), Vellore, India, 2025, 1-6. https://doi.org/10.1109/SENNET64220.2025.11135923
69. Turvey ND & Smethurst PJ (1994) Nutrient concentrations in foliage, litter and soil in relation to wood production of 7- to 15-year-old Pinus radiata in Victoria, Australia. Australian Forestry, 57(4): 157–164. https://doi.org/10.1080/00049158.1994.10676132
70. Vallejos, X., Hidalgo, S., González, B., & Neumann, P. (2025). Estimation of the Water Footprint of Wood Construction in Chile Using a Streamlined Input–Output-Based Model. Sustainability, 17(3), 1061. https://doi.org/10.3390/su17031061
71. Winkel G, Gleißner J, Pistorius T, Sotirov M & Storch S (2011). The sustainably managed forest heats up: discursive struggles over forest management and climate change in Germany. Critical Policy Studies, 5(4): 361–390. https://doi.org/10.1080/19460171.2011.628002
72. Woo J-H, Lee M-K, Chun J-H & Lee C-B. (2025). Forest Strata and Abiotic Factors Primarily Regulate Understory Species Richness Rather than Forest Type in a Temperate Forest of South Korea. Biology, 14(11):1565. https://doi.org/10.3390/biology14111565
73. Yi M, Zhang D, An Z, Cong P, Li K, Liu W & Sui K (2025). Spatiotemporal Dynamics and Drivers of Vegetation NPP in the Yanshan-Taihang Mountain Ecological Conservation Zone from 2004 to 2023. Sustainability, 17(21), 9552. https://doi.org/10.3390/su17219552
74. Yu JC, Liou WT & Chiang PN (2025). Prolonged Spring Drought Suppressed Soil Respiration in an Asian Subtropical Monsoon Forest. Forests, 16(10): 1554. https://doi.org/10.3390/f16101554
75. Zhang D & Stenger A (2014). Value and valuation of forest ecosystem services. Journal of Environmental Economics and Policy 4(2): 129–140. https://doi.org/10.1080/21606544.2014.980852

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APPLICATIONS OF THE DNA MICROARRAY TECHNIQUE

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Abstract: The completion of the sequencing of the human genome in 2001 has made it possible to carry out numerous experiments and research. Research methods are evolving from traditional molecular biology methods to methods in which multiple genes can be analyzed in a single experiment. Microarray technology can achieve the screening of thousands of DNA and protein samples simultaneously. The basic principle of microarrays is hybridization between two DNA strands. This method is widely used in various fields: genomics, proteomics, personalized medicine, vaccine screening, toxin screening, post-translational modifications, molecular diagnostics and drug discovery. In this review, the basic principles of gene expression microarrays, the types and potential applications of this technique are detailed.

Keywords: DNA microarray; drug; cancer; genetic polymorphism

Introduction

The completion of genome sequencing of many organisms has led to the evolution of research from sequencing to identifying the genes of a particular organism and their biological functions. Research methods have evolved from traditional molecular biology methods to methods in which multiple genes can be analyzed in a single experiment. The microarray technique was first used for immunological tests. But, today, different types of microarrays (DNA, proteins, tissues) can be powerful and sensitive tools for analyzing thousands of molecules in a biological system, leading to a global picture of the system under study (Howbrook et al., 2003; Templin et al., 2002).

Thus, in recent years, microarray technology has become a technology of interest in biological research, because through miniaturization, it allows the analysis and monitoring of numerous genes on a single chip, and through bioanalytical detection, it allows obtaining information about the interactions between thousands of genes simultaneously. Thus, numerous molecules can be detected simultaneously in a single experiment, using a small amount of sample, but also with high sensitivity (Howbrook et al., 2003; Templin et al., 2002; Shi et al., 2003).

Microarray technology is a valuable tool in genomic research, representing a rapid and efficient method for analyzing gene expression. Knowledge of rapid quality monitoring techniques, such as microarray technology, as well as their use and implementation, makes it possible to identify and resolve potential

problems more quickly, thus achieving the safety and efficacy of pharmaceutical products (Howbrook et al., 2003).

The aim of this paper is to identify the benefits and limitations of microarray technology, thus highlighting the utility and accessibility of this technique in the future.

DNA microarray technique – description and classification

A microarray (DNA chip or biochip) experiment involves the hybridization of an mRNA molecule to the DNA template from which it originates. The amount of mRNA bound to each site on the array indicates the expression level of the various genes. The data is collected by the system, which generates a gene expression profile. Thus, in the DNA microarray technique, numerous single-stranded DNA molecules are attached and immobilized to a solid nylon or glass surface of the chip. The samples can be represented by cDNA sequences (complementary DNA) amplified by PCR or by short DNA oligonucleotide sequences (Shin et al., 2005; Leveque et al., 2013; Chiodi et al., 2021).

Regarding the operating principle of the microarray technique, it can be schematically represented by: 1. Sample Preparation: extraction of RNA or DNA from cells of interest; reverse transcription of RNA into complementary DNA (cDNA) if necessary, using the enzyme reverse transcriptase and fluorescent labelling of DNA or cDNA samples to allow detection. 2. Hybridization: incubation of the labeled sample with the chip, where hybridization of the sample with single-stranded complementary probes fixed to the chip occurs; based on complementarity, only sequences in the sample that are complementary to the sequences on the chip will bind. 3. Washing: removal of sequences that are not specifically bound to the chip; only strongly bound sequences remain hybridized to the chip. 4. Scanning: scanning the chip to detect fluorescent signals. 5. Data analysis: visualization of the fluorescent signal intensity to determine gene expression levels and use of specialized software for data analysis (Shin et al., 2005; Leveque et al., 2013).

The basic principle of microarrays is hybridization between two DNA strands, which refers to the ability of complementary nucleic acid sequences to interact and bind specifically, by forming hydrogen bonds between complementary nitrogenous base pairs. The intensity of the fluorescent signal of the labeled target sequences depends on the strength of the hybridization and is determined by several factors: the number of complementary base pairs, the hybridization temperature, and the post-hybridization wash. The signal of a spot depends on the amount of target sample that binds to the probes present in that spot. This technique uses relative quantification, in which the intensity of a feature is compared to the intensity of the same feature under a different condition (Drmanac et al., 1998; Pollack et al., 1999; Ma and Horiuchi, 2006).

The main types of microarray technologies can be classified as in **figure 1**. (Baldi and Hatfield, 2002; LaFramboise, 2009; Arenkov et al., 2000; Limberger et al., 2017).

Applications of DNA microarray technology

Applications of microarray technology have been increasingly developed in recent years, showing the superior power of this technology for the massively parallel analysis of biological samples or the identification of genes and their functions or their mutations.

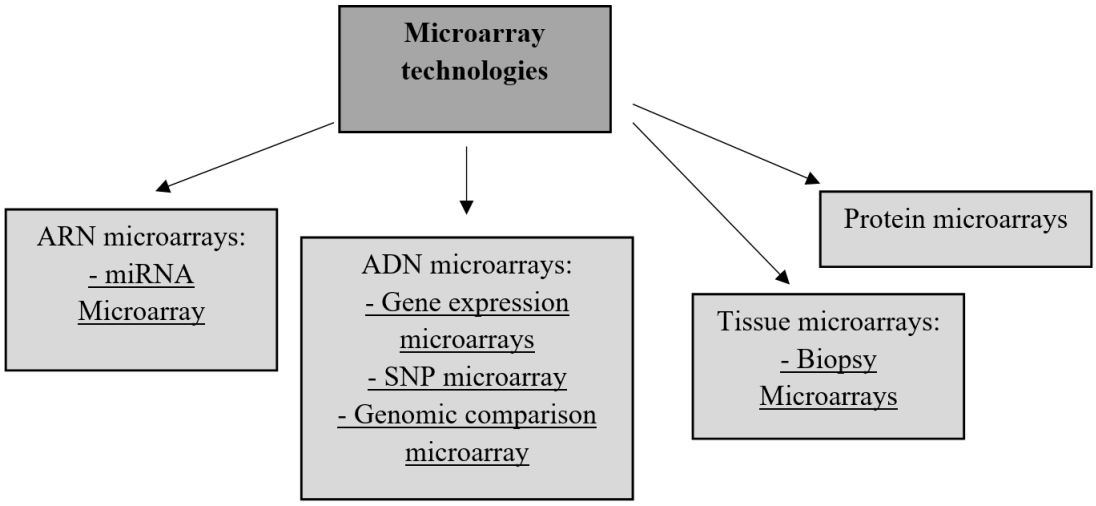

Fig. 1. Classification of the main types of microarray technologies

This method is widely used in various fields, including genomics, proteomics, personalized medicine, screening of vaccine candidates, toxin screening, post-translational modifications, molecular diagnostics, and drug discovery (Miller and Tang, 2009; Aparna and Tetala, 2023).

Drug development

Microarrays play a critical role in the discovery and development of new drugs. They are used to assess the effects of chemical compounds on gene and protein expression, identifying potential drug candidates. Gene expression studies can highlight molecular targets for therapeutic interventions and help understand the mechanisms of action of drugs (Ma and Horiuchi, 2006; Miller and Tang, 2009). To determine the therapeutic efficacy of drugs and their potential for risk, a development process involving testing of their metabolic function and toxicity occurs (Ishida et al., 2018). Some studies have shown that in vitro cell microarrays are successfully used in drug screening studies. They have a short analysis time and low cost, which reduces the need for animal testing studies. With these microarrays, specific analyses can be determined, such as the response to a drug treatment or cell-cell interactions. In addition, these microarrays can be used to determine the toxicity and side effects of pharmaceutical compounds by studying changes in gene expression in response to treatment. This allows the identification of potential safety issues at an early stage of drug development and reduces the risk of failure in later stages (Miller and Tang, 2009).

The interaction between different cell types can be understood using three-dimensional (3D) cell microarrays that can be used as an alternative to conventional two-dimensional (2D) multi-well plate assays (Gurski et al., 2010; Souza et al., 2010). It has been shown that compared to cells from native tissues and those in 3D culture conditions, cells that are cultured in 2D monolayers show significant changes in gene expression. (Gurkan et al., 2010; Pampaloni et al., 2007) Researchers can define structure-function relationships using 3D cell cultures, but they can also allow the observation of cellular events, disease progression, and response to different drugs. Microengineering and specific technologies allow the fabrication of cell-based 3D microarrays, including cell printing and surface patterning (Birgersdotter et al., 2005; Kunz-Schughart et al., 2004).

Cell printing has been shown to produce repeatable and uniform 3D cell aggregates and constructs. However, there are some difficulties with existing cell printing technologies, such as low cell viability and loss of cell functionality. However, acoustic cell printing technologies are now available that offer advantages over existing printing technologies in terms of higher cell viability and functionality (Guillemot et al., 2010; Xu et al., 2008; Jayasinghe et al., 2006).

To print cells on streptavidin-coated slides, Hart et al., (2009) used a robotic microarray placement device, with cells being placed in collagen or alginate nanodroplets. (Hart et al., 2009) Using the robotic system, these can be assembled onto a glass slide to form 3D cell microarrays. Such bioprinting platforms can be used to ensure controlled and high-yield delivery of drugs into cellular matrices. Therefore, bioprinting technology is a suitable method for the formation of 3D cellular matrices, but also for the delivery and testing of compounds (Fernandes et al., 2008; Park et al., 2010).

For successful and high-throughput drug screening, controlled drug delivery into cell microarrays is extremely important. A simple way is to load drugs using a robotic system. However, loading numerous chemicals into cell microarrays can take a long time: hours or even days. Cell viability and reproducibility of cell-drug responses can be affected by the long loading time. To efficiently deliver drugs into cell microarrays and overcome the time barrier, various methods have been developed: drug patterning, microfluidic drug loading, and aerosol sprays (Upadhyaya and Selvaganapathy, 2010; Gosalia and Diamond, 2003; Ma et al., 2005; MacBeath and Schreiber, 2000).

Due to microengineering and advances in the field (microfabrication and soft lithography), it has been possible to fabricate high-density well arrays with well sizes ranging from tens to hundreds of micrometers. In drug design, lithography has become increasingly popular due to its low cost and compatibility with a wide range of materials. The wells are loaded with cells immobilized inside these microwells, forming cell microarrays. They are used in pharmaceutical research for the identification of therapeutic targets and analysis of drug effects, as well as for the development of biomarkers and personalized therapies (Sui et al., 2013; Candela et al., 2010).

Detection and identification of bacteria and viruses

Since thousands of DNA or RNA sequences can be analyzed simultaneously with the help of microarray technology, it makes it a useful technique in microbiological diagnostics, molecular epidemiology and pathogenicity studies. Molecular methods such as PCR, real-time PCR have replaced the laborious traditional methods. However, these techniques have some limitations, such as the long time for the development and optimization of each assay, especially for multiplex PCR, real-time PCR, etc. (Kellogg et al., 2012; Fischer et al., 2008). Furthermore, many viruses are refractory to culture, and analysis by electron microscopy can be difficult depending on the type and morphological characteristics of the virus. Therefore, microarray techniques are increasingly used, with reduced analysis time and the possibility of detecting hundreds of thousands of genes in a single experiment (Donatin and Drancourt, 2012; Wang et al., 2002; Fischer et al., 2008; Peterson et al., 2010).

Many pathogenic bacterial strains vary in their ability to cause disease. Thus, techniques for detecting genes encoding virulence factors are of great importance for the identification

and characterization of bacterial pathogens. Screening pathogenic bacterial strains for genetic elements can provide important information about how virulence factors are acquired by pathogenic bacterial strains (Peterson et al., 2010).

An important application of microarray technology is the clinical diagnosis of infectious diseases (Cao et al., 2017). With the help of the microarray technique, rapid and accurate detection of bacteria and viruses from various samples: blood, urine, cerebrospinal fluid and respiratory secretions can be achieved. The technique can be used to diagnose various infections of the respiratory, gastrointestinal and central nervous system. In the case of acute respiratory infections, the simultaneous presence of several respiratory viruses can be detected: influenza virus, respiratory syncytial virus (RSV) and coronaviruses, including SARS-CoV-2. Thus, the microarray technique can be used for rapid and accurate diagnosis. The technique is also used for the detection of antimicrobial resistance, for the detection of *Neisseria meningitidis*, for the identification of mutations, but also for the adaptation of vaccination and treatment strategies (Damin et al., 2021; Wang et al., 2020; Asmare and Erkihun, 2023).

Microarray technology is also used in pathogenicity studies to better understand the molecular mechanisms by which bacteria and viruses cause disease. The pathogenesis of infections, as well as the genes and signaling pathways involved in virulence, can be determined by comparing the gene expression profiles of different pathogens. Thus, microarray technology can be used to identify virulence genes in pathogenic bacteria, such as *Staphylococcus aureus*. Such studies have led to the discovery of new strategies for the development of antibiotics and vaccines, leading to improved mechanisms to combat bacterial infections (Moneche and Ehricht, 2005; Zhu et al., 2007).

Detection of genes involved in cancer

Cancer is a complex disease characterized by genetic and epigenetic changes that lead to uncontrolled cell division, playing an important role in its early diagnosis. The gene expression of a cell determines how it manifests itself, its function, but also its response to different stimuli. With the help of gene expression profiles, regulatory mechanisms, but also cellular functions can be determined. For the early detection of cancer, the commonly used methods (classical imaging methods and morphological analysis of tissues or cells) have certain limitations. The microarray technique is a modern technique that allows the understanding of the molecular mechanisms of cancer, early diagnosis, prognosis and the development of targeted therapies. Microarray technology has thus become an essential technique in cancer research, allowing the analysis of gene expression and the identification of genetic variations (Schena, 2000; Wikman et al., 2000).

Oligonucleotide microarrays have been used as a method for rapid analysis of mutations in selected gene sequences and are effective in sequence analysis, genetic disease diagnosis, and gene polymorphism studies. cDNA microarrays are commonly used for gene expression analysis, and their use is relatively easy (Wikman et al., 2000; Yershov et al., 1996). Researchers hypothesized that the genes expressed by the two tumor types (BRCA1 mutation carriers vs. BRCA2 mutation carriers) are distinct. An analysis of the variation between gene expression levels and sample genotypes identified 176 genes that were differentially expressed in BRCA1-mutated tumors and BRCA2-mutated tumors. Yuan et al., (2003) used cDNA microarrays to identify gene expression patterns among

colorectal cancer cell lines and to directly compare lines with and without microsatellite instability. Several differential expression patterns were identified (Hedenfalk et al., 2001; Yuan et al., 2003). Characterization of DNA copy number is important for both the basic understanding of cancer and its diagnosis. cDNA microarrays have been widely used to characterize human gene expression variation (Gupta et al, 1999; Pollack et al., 1999).

Microarray-based expression profiling allows the identification of gene families as well as important molecular and cellular events that may be essential in complex processes such as metastasis. In the future, practical applications relate to the diagnostic and prognostic organization of patients. To determine the mechanisms of action of drugs, as well as their sensitivity and toxicity, the microarray technique can be used in clinical trials. Thus, microarrays are useful for developing studies of molecular taxonomy of cancer, including the organization of cancer types into groups, based on gene expression profiles. Therefore, the microarray technique can be used for: molecular phenotyping, the study of gene function, functional genomics and pharmacogenomics (Pollack et al., 1999).

Studies have illustrated the efficacy of microarrays in oral cancer, through gene expression analysis. Early diagnosis and management of oral cancer is correlated with increased survival (Alevizos et al., 2001).

Because cancers result from the accumulation of many genetic and epigenetic alterations, microarray technologies are important in cancer research although there are some disadvantages to the routine use of microarrays. The cost of microarray experiments can be quite high due to the need to standardize the technique and to develop data analysis methods that allow comparison of data between different research groups. However, microarray techniques are considered important methods in cancer research. Using oligonucleotide microarrays, early diagnosis of cancer can be achieved, and gene expression profiling can be used to obtain prognosis after chemotherapy or radiotherapy (Kim et al., 2004).

Genetic polymorphism detection

Genetic polymorphisms refer to DNA sequence variations that occur within a population and that can determine different phenotypic traits, susceptibility to disease, and response to different treatments. The identification and characterization of genetic polymorphisms are important for understanding personalized medicine and genetic association studies. Microarray technology has revolutionized the detection of polymorphisms, providing a rapid, accurate, and high-throughput method for analyzing genetic variation (LaFramboise, 2009).

In clinical diagnosis, polymorphism-based microarray analysis allows the detection of copy number variations (CNVs). A single nucleotide polymorphism (SNP) is a variation at a single position in a DNA sequence between individuals. It is a common genetic variation in which a single nucleotide (A, C, G, or T) is replaced by another nucleotide at a specific locus in the DNA sequence. Each SNP locus in the genome can have up to four versions, one for each nucleotide (Arsham et al., 2017; Auton et al., 2013; Palmisano et al., 2005).

To date, there are over 200,000 single nucleotide polymorphisms (SNPs) associated with traits or diseases, according to the Genome-Wide Association Studies (GWAS) catalog, which is a database of genome-wide association studies. However, not all SNPs associated with diseases are directly responsible for the development of a disease. Some SNPs are linked to other genetic variants that are directly involved in the occurrence of

the disease. In addition, the effect of SNPs on disease risk varies depending on different factors, such as: environment, lifestyle and interactions with other genes (Uffelmann et al., 2021).

The microarray technique also has some limitations, as it cannot ensure the detection of unknown SNP mutations. Moreover, the use of large data sets requires the use of complex analysis and calculation methods, the cost of chip preparation, reagents and equipment being quite high (Ahmad and Iqbal, 2012; Iwamoto et al., 2007; Hirchhorn and Daly, 2005).

A frequently used application of gene expression analysis is the scanning of the entire genome to identify SNPs associated with phenotypic traits and various diseases. SNP microarrays are important in these analyses, and allow the simultaneous analysis of millions of genetic variations in large population samples. These studies have led to the discovery of new genes and molecular pathways involved in complex diseases, such as diabetes, cardiovascular disease, and cancer (Hirchhorn and Daly, 2005; Shastry, 2006).

Identification of genetic polymorphisms by microarray is important for the development of personalized medicine. Genetic analysis of SNPs can provide important information about individuals' susceptibility to certain diseases, drug response, and risk of developing adverse reactions to certain drugs. For example, microarray tests can identify variants in the CYP2C19 gene to guide treatment with anticoagulant drugs such as clopidogrel. The importance of these genetic identifications for developing personalized treatments, improving therapeutic efficacy, and reducing adverse reactions is also emphasized (Shastry, 2006).

Microarray techniques are also used in the diagnosis of genetic diseases by detecting pathogenic mutations and chromosomal variations. CGH microarrays can identify CNVs associated with genetic syndromes, such as DiGeorge syndrome and Williams-Beuren syndrome. These techniques can accurately identify chromosomal deletions in the 22q11.2 region, the characteristic region of DiGeorge syndrome, which contains several genes important for normal development, the loss of which can lead to the symptoms associated with the syndrome. Microarray techniques are being developed to allow precise detection of the exact size and location of the deletion. SNP microarrays can also detect mutations associated with monogenic diseases such as cystic fibrosis and hemophilia (Shastry, 2006; McDonald-McGinn and Sullivan, 2011).

Conclusions

Microarray technology is revolutionizing the way biological research is conducted, as it allows the simultaneous analysis of thousands of uniquely identified genes in a single experiment. Various variants of microarrays have been successfully used, alone or in combination, in biological research, medical diagnostics, drug discovery and development, and toxicogenomics, leading to an acceleration of progress in current fields.

Although microarray techniques can be improved technologically, the results and conclusions of published studies have clearly demonstrated the utility and power of these miniaturized tools. Microarray-based clinical diagnostic tools can represent a basis for personalized medicine.

Although microarrays are primarily research techniques, microarray-based approaches are rapid and flexible.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Ahmad A, Iqbal MA (2012) Significance of genome-wide analysis of copy number alterations and UPD in myelodysplastic syndromes using combined CGH-SNP arrays. Curr Med Chem. 19(22): 3739-3747. doi: 10.2174/092986712801661121
2. Alevizos I, Mahadevappa M, Zhang X, Ohyama H, Wong DT (2001) Oral cancer in vivo gene expression profiling assisted by laser capture microdissection and microarray analysis. Oncogene 20(43): 6196-6204. doi: 10.1038/sj.onc.1204685
3. Arenkov P, Kukhtin A, Gemmell A, Voloshchuk S, Chupeeva V, Mirzabekov A (2000) Protein Microchips: Use for Immunoassay and Enzymatic Reactions. Anal Biochem. 278(2): 123-131. doi: 10.1006/abio.1999.4363
4. Arsham MS, Barch MJ, Lawce HJ (2017) The AGT Cytogenetics Laboratory Manual. 4th Edition, NJ: John Wiley & Sons, Inc., pp 45-46.
5. Aparna GM, Tetala KKR (2023) Recent Progress in Development and Application of DNA, Protein, Peptide, Glycan, Antibody, and Aptamer Microarrays. Biomolecules 13(4): 602. https://doi.org/10.3390/biom13040602
6. Asmare Z, Erkihun M (2023) Recent Application of DNA Microarray Techniques to Diagnose Infectious Disease. Pathology and Laboratory Medicine International 15: 77-82. doi: https://doi.org/10.2147/PLMI.S424275
7. Auton A, Li RY, Kidd J, Oliveira K, Boyko AR (2013) Genetic recombination is targeted towards gene promoter regions in dogs. PLoS Genet. 9(12): e1003984. doi: 10.1371/journal.pgen.1003984
8. Baldi P, Hatfield GW (2002) DNA Microarrays and Gene Expression: From Experiments to Data Analysis and Modeling, Cambridge University Press, pp 2-90. https://assets.cambridge.org/97805218/00228/frontmatter/9780521800228_frontmatter.pdf
9. Birgersdotter A, Sandberg R, Ernberg I (2005) Gene expression perturbation in vitro - A growing case for three-dimensional (3D) culture systems. Semin Cancer Biol. 15(5): 405-412. doi: 10.1016/j.semcancer.2005.06.009
10. Candela M, Consolandi C, Severgnini M, Biagi E, Brigidi P (2010) High taxonomic level fingerprint of the human intestinal microbiota by Ligase Detection Reaction–Universal Array approach. BMC Microbiol. 10(116). doi: 10.1186/1471-2180-10-116
11. Cao J, Gao S, Chen J, Zhu B, Wang P (2018) The preparation and clinical application of diagnostic DNA microarray for the detection of pathogens in intracranial bacterial and fungal infections. Experimental and Therapeutic Medicine 16: 1304-1310. doi: 10.3892/etm.2018.6312
12. Chiodi E, Damin F, Sola L, Ferraro L, Chiari M (2021) A reliable, label free quality control method for the production of DNA microarrays with clinical applications. Polymers (Basel) 13(3): 340. doi: 10.3390/polym13030340
13. Damin F, Galbiati S, Gagliardi S, Cereda C, Chiari M (2021) Covid Array: A Microarray-Based Assay with High Sensitivity for the Detection of Sars-Cov-2 in Nasopharyngeal Swabs. Sensors (Basel). 21(7): 2490. doi: 10.3390/s21072490
14. Donatin E, Drancourt M (2012) DNA Microarrays for the Diagnosis of Infectious Diseases, Med Mal Infect. 42(10): 453-459. doi: 10.1016/j.medmal.2012.07.017
15. Drmanac S, Kita D, Labat I, Hauser B, Drmanac R (1998) Accurate Sequencing by Hybridization for DNA Diagnostics and

Individual Genomics. Nat Biotechnol. 16(1): 54-58. doi: 10.1038/nbt0198-54
16. Fernandes TG, Kwon S-J, Lee M-Y, Clark DS, Dordick JS (2008) On-chip, cell-based microarray immunofluorescence assay for high-throughput analysis of target proteins. Anal Chem. 80(17): 6633-6639. doi: 10.1021/ac800848j
17. Fischer K, Urisman A, DeRisi J (2008) Diagnostic Techniques: Microarrays, Encyclopedia of Virology pp 14-18. doi: 10.1016/B978-012374410-4.00704-4
18. Gosalia DN, Diamond SL (2003) Printing chemical libraries on microarrays for fluid phase nanoliter reactions. Proc Natl Acad Sci U S A. 100(15): 8721–8726. doi: 10.1073/pnas.1530261100
19. Guillemot F, Souquet A, Catros S, Guillotin B, Amedee J (2010) High-throughput laser printing of cells and biomaterials for tissue engineering. Acta Biomater. 6(7): 2494-2500. doi: 10.1016/j.actbio.2009.09.029
20. Gurkan UA, Gargac J, Akkus O (2010) The Sequential Production Profiles of Growth Factors and their Relations to Bone Volume in Ossifying Bone Marrow Explants. Tissue Eng Part A. 16(7): 2295-2306. doi: 10.1089/ten.TEA.2009.0565
21. Gurski LA, Petrelli NJ, Jia X, Farach-Carson MC (2010) 3D Matrices for Anti-Cancer Drug Testing and Development, Oncology Issues 25(1): 20-25. https://doi.org/10.1080/10463356.2010.11883480
22. Gupta PK, Roy JK, Prasad M (1999) DNA chips, microarray and genomics. Curr Sci. 77(7): 875-884. https://eurekamag.com/research/003/397/003397785.php
23. Hart T, Zhao A, Garg A, Bolusani S, Marcotte EM (2009) Human cell chips: adapting DNA microarray spotting technology to cell-based imaging assays. PLoS One. 4(10): e7088. doi: 10.1371/journal.pone.0007088
24. Hedenfalk I, Duggan D, Chen Y, Radmacher M, Sauter G (2001) Gene-expression profiles in hereditary breast cancer. N Engl J Med. 344(8): 539-548. doi: 10.1056/NEJM200102223440801
25. Hirschhorn JN, Daly MJ (2005) Genome-wide association studies for common diseases and complex traits. Nat Rev Genet. 6(2): 95-108. doi: 10.1038/nrg1521
26. Howbrook ND, van der Valk AM, O'Shaughnessy MC, Sarker DK, Baker SC, Lloyd A W (2003) Developments in microarray technologies. Drug Discov. Today 8(14): 642-651. doi: 10.1016/s1359-6446(03)02773-9
27. Ishida S, Horiuchi S, Kuroda Y, Fujii R, Kanda Y (2018) DNA microarray analysis on characteristics of hepatocyte-like cells derived from human iPS cells for the application to the cell based drug safety tests. Toxicology Letters 295(1): S225-S226 https://doi.org/10.1016/j.toxlet.2018.06.957
28. Iwamoto K, Bundo M, Ueda J, Nakano Y, Kato T (2007) Detection of chromosomal structural alterations in single cells by SNP arrays: a systematic survey of amplification bias and optimized workflow. Plos One 2(12): e1306. doi: 10.1371/journal.pone.0001306
29. Jayasinghe SN, Qureshi AN, Eagles PAM (2006) Electrohydrodynamic jet processing: an advanced electric-field-driven jetting phenomenon for processing living cells. Small 2(2): 216-219. doi: 10.1002/smll.200500291
30. Kellogg CA, Piceno YM, Tom LM, DeSantis TZ, Andersen GL (2012) PhyloChip microarray comparison of sampling methods used for coral microbial ecology. *J* Microbiol Methods. 88(1): 103-109. doi: 10.1016/j.mimet.2011.10.019

31. Kim I-J, Kang HC, Park J-G (2004) Microarray Applications in Cancer Research, Cancer Res Treat. 36(4): 207-213. doi: 10.4143/crt.2004.36.4.207
32. Kunz-Schughart LA, Freyer JP, Hofstaedter F,Ebner R (2004) The use of 3-D cultures for high-throughput screening: the multicellular spheroid model. J Biomol Screen. 9(4): 273-285. doi: 10.1177/1087057104265040
33. LaFramboise T (2009) Single nucleotide polymorphism arrays: a decade of biological, computational and technological advances. Nucleic Acids Res. 37(13): 4181-4193. doi: 10.1093/nar/gkp552
34. Lěvěque N, Renois F, Andreoletti L (2013) The Microarray Technology: Facts and Controversies. Clin Microbiol Infect. 19(1): 10-14. doi: 10.1111/1469-0691.12024
35. Limberger KA, Bogatyreva L, Todorova R, Herde B, Aumann K (2017) Tissue Microarray Technique Is Applicable to Bone Marrow Biopsies of Myeloproliferative Neoplasms. Histochem Cell Biol. 147(1): 75-82. doi: 10.1007/s00418-016-1476-x
36. Ma H, Horiuchi KY (2006) Chemical Microarray: A New Tool for Drug Screening and Discovery. Drug Discov Today. 11(13-14): 661-668. doi: 10.1016/j.drudis.2006.05.002
37. Ma H, Horiuchi KY, Wang Y, Kucharewicz SA, Diamond SL (2005) Nanoliter homogenous ultra-high throughput screening microarray for lead discoveries and IC50 profiling, Assay Drug Dev Technol. 3(2): 177-187. doi: 10.1089/adt.2005.3.177
38. MacBeath G, Schreiber SL (2000) Printing proteins as microarrays for high-throughput function determination, Science 289(5485): 1760-1763. doi: 10.1126/science.289.5485.1760
39. McDonald-McGinn DM, Sullivan KE (2011) Chromosome 22q11.2 deletion syndrome (DiGeorge syndrome/velocardio facial syndrome). Medicine (Baltimore) 90(1): 1-18. doi: 10.1097/MD.0b013e3182060469
40. Miller MB, Tang Y-W (2009) Basic Concepts of Microarrays and Potential Applications in Clinical Microbiology. Clin Microbiol Rev. 22(4): 611–633. doi: 10.1128/CMR.00019-09
41. Monecke S, Ehricht R (2005) Rapid genotyping of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates using miniaturized oligonucleotide arrays, Clin Microbiol Infect. 11(10): 825-833. doi: 10.1111/j.1469-0691.2005.01243
42. Palmisano GL, Delfino L, Fiore M, Longo A, Ferrara GB (2005) Single Nucleotide Polymorphisms Detection Based on DNA Microarray Technology: HLA as a Model. Autoimmun Rev. 4(8): 510-514. doi: 10.1016/j.autrev.2005.04.011
43. Pampaloni F, Reynaud EG, Stelzer EHK (2007) The third dimension bridges the gap between cell culture and live tissue, Nat Rev Mol Cell Biol. 8(10): 839-845. doi: 10.1038/nrm2236
44. Park JH, Chung BG, Lee WG, Kim J, Khademhosseini A (2010) Microporous Cell-Laden Hydrogels for Engineered Tissue Constructs. Biotechnol Bioeng. 106(1): 138-148. doi: 10.1002/bit.22667
45. Peterson G, Bai J, Nagaraja TG, Narayanan S (2010) Diagnostic Microarray for Human and Animal Bacterial Diseases and Their Virulence and Antimicrobial Resistance Genes, J Microbiol Methods. 80(3): 223-230. doi: 10.1016/j.mimet.2009.12.010
46. Pollack JR, Perou CM, Alizadeh AA, Eisen MB, Brown PO (1999) Genome-wide analysis of DNA copy-number changes using cDNA microarrays. Nat Genet. 23(1): 41-46. doi: 10.1038/12640

47. Shastry BS (2006) Pharmacogenetics and the concept of individualized medicine, Pharmacogenomics J. 6(1): 16-21. doi: 10.1038/sj.tpj.6500338
48. Schena M (2000) Microarray Biochip Technology, Eaton Pub., pp 1-18. https://archive.org/details/microarraybiochi0000unse/page/n351/mode/2up
49. Shi L, Hu W, Su Z, Lu X, Tong W (2003) Microarrays: Technologies and Applications. Volume 3. Applied Mycology and Biotechnology, ScienceDirect, pp 271-293. https://doi.org/10.1016/S1874-5334(03)80016-3
50. Shin Y, Kitayama A, Koide T, Peiffer DA, Cho KWY (2005) Identification of neural genes using Xenopus DNA microarrays. Dev Dyn. 232(2): 432-444. doi: 10.1002/dvdy.20229
51. Sui H-T, Wang D-Y, Shu Y-L (2013) Development of multi-pathogen detection techniques for respiratory viruses. Bing Du Xue Bao. 29(2): 238-244. PMID: 23757859
52. Souza GR, Molina JR, Raphael RM, Ozawa MG, Pasqualini R (2010) Three-dimensional tissue culture based on magnetic cell levitation. Nat Nanotechnol. 5(4): 291-296. doi: 10.1038/nnano.2010.23
53. Templin MF, Stoll D, Schrenk M, Traub PC, Vohringer CF, Joos TO (2002) Protein Microarray Technology. Drug Discov. Today 7(15): 815-822. doi: 10.1016/s1359-6446(00)01910-2
54. Uffelmann E, Huang QQ, Munung NS, de Vries J, Posthuma D (2021) Genome-Wide Association Studies. Nat Rev Methods Primers 1: 1-21. doi: 10.1038/s43586-021-00056-9
55. Upadhyaya S, Selvaganapathy PR (2010) Microfluidic devices for cell based high throughput screening, Lab on a Chip 10(3): 341-348. doi: 10.1039/b918291h
56. Wang D, Coscoy L, Zylberberg M, Avila PC, DeRisi JL (2002) Microarray-based detection and genotyping of viral pathogens. Proc Natl Acad Sci U S A. 99(24): 15687-15692. doi: 10.1073/pnas.242579699
57. Wang H, Wu X, Zhang X, Hou X, Yu X (2020) SARS-CoV-2 Proteome Microarray for Mapping COVID-19 Antibody Interactions at Amino Acid Resolution. ACS Cent Sci. 6(12): 2238-2249. doi: 10.1021/acscentsci.0c00742
58. Wikman FP, Lu ML, Thykjaer T, Olesen SH, Orntoft TF (2000) Evaluation of the performance of a p53 sequencing microarray chip using 140 previously sequenced bladder tumor samples. Clin Chem. 46 (10): 1555-1561. PMID: 11017932 https://pubmed.ncbi.nlm.nih.gov/11017932/
59. Xu T, Kincaid H, Atala A, Yoo JJ (2008) High-throughput production of single-cell microparticles using an inkjet printing technology. J Manuf Sci Eng. 130(2): 021017. https://doi.org/10.1115/1.2903064
60. Yershov G, Barsky V, Belgovskiy A, Kirillov E, Mirzabekov A (1996) DNA analysis and diagnostics on oligonucleotide microchips. Proc Natl Acad Sci USA (PNAS) 93(10): 4913-4918. doi: 10.1073/pnas.93.10.4913
61. Yuan Z, Kent TS, Weber TK (2003) Differential expression of DOC-1 in microsatellite-unstable human colorectal cancer. Oncogene 22(40): 6304-6310. doi: 10.1038/sj.onc.1206609
62. Zhu L-X, Zhang Z-W, Wang C, Yang H-W, Cheng J (2007) Use of a DNA microarray for simultaneous detection of antibiotic resistance genes among Staphylococcal clinical isolates. J Clin Microbiol. 45(11): 3514-3521. doi: 10.1128/JCM.02340-06

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STUDY ON WILD BLACK RASPBERRY (*RUBUS NIVEUS*) FRUIT: VALORIZATION OF ITS POTENTIAL TO NEUTRALIZE FREE-RADICALS AND REACTIVE OXYGEN SPECIES (ROS) AND ITS PHOTOSTABILITY UNDER SOLAR LIGHT

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Abstract: The present study evaluated the photostability of *Rubus niveus* fruit using a solar simulator. Since degradation under solar irradiation is a key indicator of stability capacity, photostability assays are fundamental to evaluating the preponderance of *R. niveus* extract for biological applications involving light. Additionally, the quenching and scavenging capacity against different types of free radicals was assessed. Ethanolic extracts were prepared from frozen black raspberries. Quenching and scavenging activity were measured using a series of analytical procedures. These included UV-Vis spectroscopic analysis, photostability assessment using solar radiation spectrum (with solar simulator), chemiluminescence, and DPPH assays. Reactions with singlet oxygen ($^1O_2$) and galvinoxyl radical were also employed to evaluate scavenging capacity. The results revealed that *R. niveus* is a source of anthocyanins (128.68±0.85 mg/lt and 48.19±0.31 mg/100 g of fresh weight) and exhibits remarkable photostability under solar light, with minimal degradation at its absorption wavelength. Its photostability was comparable to that of common sunscreen filters (Neo Heliopan Type Ma and Neo Heliopan Type OS). Furthermore, a noteworthy antioxidant capacity was demonstrated, effectively reducing reactive oxygen species (ROS) and free radicals, with significant scavenging activity comparable to that of vitamin C in some assays. Particularly, the extract displayed superior scavenging activity against highly reactive hydroxyl radicals compared to vitamin C (86.82% vs. 55.13%). These results suggest that the ethanolic extract of *R. niveus* is photostable and has the capacity to scavenge and neutralize different types of dark- and light-induced reactive oxygen species. Further *in vitro* and *in vivo* research is recommended to validate the potential of *R. niveus* compounds as natural ingredients in cosmetic and nutraceutical products.

Keywords: antioxidant, photostability, fruit, raspberry, light, free-radicals

Introduction

The *Rubus niveus*, also known as the Mysore raspberry or wild black raspberry, belongs to the Rosaceae family. Originally from Asia, it has spread to various parts of the world due to its ability to adapt to different climates and altitudes. It is considered an invasive species because it grows rapidly in regions where it is cultivated due to its ability to spread easily through various reproduction mechanisms. In addition, the fruits of *R. niveus* are edible and contain many seeds that contribute to the reproduction of this plant

(Pancholi and Rana). The fruits are also used for human consumption and have high medicinal value (Li et al., 2015; Ahmad et al., 2015).

Research in phytochemistry has shown that phenolic compounds have significant anti-inflammatory and antioxidant activity. This activity is essential for reducing oxidative stress and its consequences, such as the development of cancerous tumors, heart problems, and other chronic diseases (a.Hertog et al., 1993; b. Hertog et al., 1993). Thus, scientific research in this field is particularly relevant because it provides *in vitro* tests that can establish possible links between the antioxidant activity of different plant sources and the prevention of common human diseases. It is well known that some natural antioxidant compounds are involved in photoprotection mechanisms, reducing skin damage and aging (Silva et al., 2016; Khoo et al., 2017).

Various scientific studies have reported on the pharmacological value of the *R. niveus* plant in both *in vitro* and *in vivo* settings (Chiluisa et al., 2017; Parimelazh et al., 2013; Pancholi and Rana, 2020; Badhani et al., 2015; Nesello et al., 2017). For instance, extracts from this species have been found to prevent diseases such as diabetes, obesity, hypertension, cancer, and other conditions, as well as common microbial infections. For this reason, antibacterial, anti-inflammatory, and antioxidant properties have been attributed to *R. niveus*, due to the wide variety of polyphenolic compounds, such as anthocyanins and catechins, which are beneficial to human health. Thus, *R. niveus* fruits have been shown to be relevant not only to the biomedical field, but also to nutrition and ethnomedicine (Pancholi and Rana, 2020; Ahmad et al., 2015; Muniyandi et al., 2019).

However, there is a lack of information regarding the quenching and scavenging activity of the ethanolic extract of black raspberry fruit (*R. niveus*), as well as its behavior under visible and ultraviolet irradiation. It is important to acknowledge the scarcity of research focused on the quenching, scavenging and photostability potential of *R. niveus* fruit, given the emphasis placed on the properties of other plant parts, such as roots and stems (Parimelazh et al., 2013; Badhani et al., 2015; Nesello et al., 2017). Consequently, emphasizing the protective capacity and nutritional and health-promoting potential of *R. niveus* fruit is fundamental, regardless of preconceived notions about its origin and biological characteristics (Bachheti et al., 2023). Furthermore, it should be noted that chemiluminescence assays have not been utilized in previous research on the antioxidant and scavenging activity of *R. niveus*. This is a notable observation, given the reliability and high performance of these techniques in assessing antioxidant activity of foods and raw materials (Zvereva and Zhmurova, 2023; Da Silva Mendonça et al., 2022).

This research offers a comprehensive understanding of the photochemical behaviour (photostability) exhibited by the compounds present in *R. niveu*s extract and their ability to neutralize reactive oxygen species (ROS) and free-radicals. The experimental approach utilizes UV-Vis and fluorescence studies, as well as DPPH (2,2-diphenyl-1-picrylhydrazyl) and chemiluminescence methods (which have not previously been employed for *R. niveus*). The study also examines the reactions with the galvinoxyl radical and singlet oxygen. In this sense, the objective of this study is to highlight the photostability and antioxidant properties of *R. niveus* fruit.

Materials and Methods

Reagents and equipment

A Luzchem L2C-4V solar simulator, Perkin Elmer Lambda 35 spectrometer, FL 6500 Fluorescence Spectrophotometer, Luminoskan Ascent Luminometer, Ethanol

75% (Sigma-Aldrich, HPLC grade), Neolipan type Ma (menthyl anthranilate), Neoheliopan type OS (Octisalate, 2-Ethylhexyl salicylate), DPPH radical (2,2-diphenyl-1-picrylhydrazyl, Sigma-Aldrich), Ascorbic acid (Sigma-Aldrid), Galvinoxyl radical (Sigma), Rose Bengal (Aldrich), sodium acetate (CH_3COONa), potassium chloride (KCl), hydrogen peroxide (H_2O_2, Aldrich), Potassium ferricyanide ($K_3[Fe(CN)_6]$).

Plant material

The *Rubus niveus* fruit was picked in Los Teques, Miranda State, Venezuela, at an elevation of 1,200 meters. Mature, dark purple fruits were collected and stored at -10°C.

It should be noted that the *R. niveus* plant used in this study was botanically certified by the Ecology Center of the Venezuelan Institute of Scientific Research (IVIC).

UV-Vis Spectrum

For the UV-Vis spectrum, 1 ml of the concentrated extract was dissolved in 3 ml of ethanol. The spectrum was recorded using a Perkin Elmer Lambda 35 spectrometer.

Monomeric anthocyanins quantification

The pH-differential method employed by (Lee et al., 2005) was used to quantify the total monomeric anthocyanins in black raspberry extract. One milliliter of the extract was diluted in three milliliters of the respective buffer (sodium acetate or potassium chloride), and three consecutive measurements of the solution were taken at the corresponding wavelength. ΔAbs values were obtained for each measurement, and the total anthocyanin values in milligrams per liter (mg/l) were calculated using the following formula:

$$Anthocyanin\ content\ (mg/l) = \frac{\Delta Abs\ x\ MW\ x\ DF\ x\ 1000}{\varepsilon\ x\ l}$$

(1)

Where ΔAbs = absorbance difference; MW is the molecular weight of cyanidin-3-glucoside (449.2 g/mol); DF is the dilution factor (in this case 4); l is the optical path length of the UV-Vis quartz cell; ε is the molar extinction coefficient of cyanidin-3-glucoside (26.900). These measurements were taken by triplicate. Note that the expression "ε x l" is the molar extinction coefficient multiplied by the path length of the UV-Vis quartz cell (1 cm).

On the other hand, in order to compare anthocyanin content values reported in previous research, the total anthocyanin value was calculated in milligrams per 100 grams of fresh weight (mg/100 g) using the following formula:

$$Anthocyanin\ content\ (mg/100\ g) = \frac{\Delta Abs\ x\ MW\ x\ DF\ x\ V\ (lts)\ x\ 1000}{\varepsilon\ x\ l\ x\ m(grams\ of\ sample)} x\ 100$$

(2)

Where: ΔAbs = Absorbance difference; MW= Molecular weight of cyanidin-3-glucoside (449.2 g/mol); V = Final volume of the extract (liters); m = Mass of the sample (grams); l= Path length of the UV-Vis quartz cell (1 cm); DF = Dilution factor (4 in this case); ε = Molar extinction coefficient of cyanidin-3-glucoside (26,900). These measurements were taken by triplicate. Note that "ε x l x m" is the molar extinction coefficient multiplied by the path length of the UV-Vis quartz cell (1 cm) and the mass of the sample.

Photostability assays

To evaluate the photostability of the black raspberry extract, 2 ml of the concentrated

ethanolic extract was mixed with 4 ml of distilled water. Absorbance changes were measured using a spectrophotometer after irradiating the mixture for 15 minutes with a Luzchem L2C-4V solar simulator (visible light range). The extract was irradiated in a quartz flask, and the respective changes in absorbance were measured using quartz cells for UV-visible spectrophotometry

Reactions with singlet oxygen ($^1O_2$)

The reaction of black raspberry ethanolic extract with singlet oxygen was measured by observing the decrease in absorbance of the extract under visible irradiation (227 LUX; 1.88 W/m²) and in the presence of a type II photosensitizer (Rose Bengal). To perform this experiment, a solution was prepared with 2 ml of black raspberry extract and 2 ml of distilled water, to which 50 microliters of Rose Bengal (~$2x10^{-4}$ M) were added. The total irradiation time was 3 hours, and changes in absorbance were measured in the UV-Vis spectrophotometer after 1 hour and 2 hours of irradiation.

Chemiluminescence assays

To assess the neutralization activity of hydroxyl radicals and peroxides by *Rubus niveus* fruit, chemiluminescence experiments were conducted, based on the reaction of luminol with ROS (Vargas et al., 2004). For this purpose, 10 microliters of the antioxidant (ethanolic extract of black raspberry and vitamin C for comparison) and 6 microliters of hydrogen peroxide (~3.5 mM) were taken. The intensity of chemiluminescent light was measured in a Luminoskan Ascent Luminometer every 10 seconds for a total time of 40 seconds, after the automatic injection of luminol (15 microliters). Subsequently, these same tests were carried out but in the presence of ferrous iron with the addition of 15 microliters of $K_3[Fe(CN)_6]$ (~40 nM), in order to generate hydroxyl radicals and study the scavenging capacity of these radicals by the *R. niveus* extract.

The percentage of inhibition of H_2O_2 and OH radicals was calculated using the following formula:

$$\%\ inhibition = \frac{1\ control - 1\ sample}{1\ control}\ x\ 100 \quad (3)$$

The total percentage of inhibition was obtained by comparing the samples studied to the control by measuring the intensity of the light of the samples and the control at every point and applying formula 3). The final percentage of inhibition reported was the mean of all inhibition percentages calculated at each point.

Radical scavenging activity

The radical scavenging capacity was studied using galvinoxyl and DPPH radicals by UV-Vis spectrophotometry, using the methods of (Bobinaitė et al., 2011; Gulcin and Alwasel, 2023) as references. For the DPPH radical assays, the free radical scavenging potential (RSC) was calculated 30 minutes after reaction with *Rubus niveus* extract (100 microliters) with 3 milliliters of DPPH radical (~10^{-4} M). Three consecutive measurements (triplicate) were taken and compared with the RSC_{30} value of vitamin C. The RSC of berry extracts was expressed as a percentage of inhibition of the DPPH radical, according to the following formula:

$$RSC = \frac{Absorbance\ of\ Blank\ Solution\ -\ Absorbance\ of\ Sample}{Absorbance\ of\ Blank\ Solution} x100\% \quad (4)$$

On the other hand, the reaction of black raspberry extract with the galvinoxyl radical was carried out using a solution of the same at a concentration of approximately 10^{-4} M. The titrations were performed with 3 ml of this solution, with 3 successive additions of 15

microliters of pure *R. niveus* extract. The reaction was carried out using UV-Vis spectrophotometry and fluorescence (using excitation spectrum).

Note that RSC was calculated after 30 minutes of reaction because it is a standardized method stipulated in the bibliography. Most RSC values reported in previous research were calculated using this time frame.

Statistical Analysis

To validate the results and distinguish the neutralizing effects in the chemiluminescence and DPPH assays, statistical analyses were performed using unpaired t-test with statistical analysis free-software (GraphPad). This analysis determined if there were significant differences between the compounds being compared, in this case, black raspberry extract and vitamin C.

Results and Discussions

Figure 1 shows a characteristic absorption spectrum of black raspberry ethanol extracts with a band at 543 nm, which is the typical wavelength of anthocyanin molecules according to references (Dangles and Fenger, 2018; Aguilera-Otíz et al., 2011). Meanwhile, experiments to quantify the anthocyanins in *Rubus niveus* extract using the differential pH method yielded a result of 128.68±0.85 mg/L of total anthocyanins (0.85 being the standard deviation of the measurements taken in triplicate). **Figure 2** show the photostability tests of the ethanolic extract of *R. niveus* solar irradiation (using solar simulator). This value is equivalent to 48.19±0.31 mg/100 g of fresh weight.

Figure 3 shows the activity of *R. niveus* extract against H_2O_2 and its comparison with vitamin C. **Figure 3.a** shows the decrease in chemiluminescent light intensity when *R. niveus* extract and vitamin C are added, compared to the H_2O_2 control. **Figure 3.b** shows the percentage inhibition of H_2O_2 according to the results shown in **figure 3.a**.

To evaluate the activity of the ethanolic extract of *R. niveus* against ·OH radicals, a chemiluminescence experiment was carried out, induced by the addition of ferrous iron. The results in **figure 4** show the intensity of chemiluminescent light detected after the addition of the antioxidants studied for comparison.

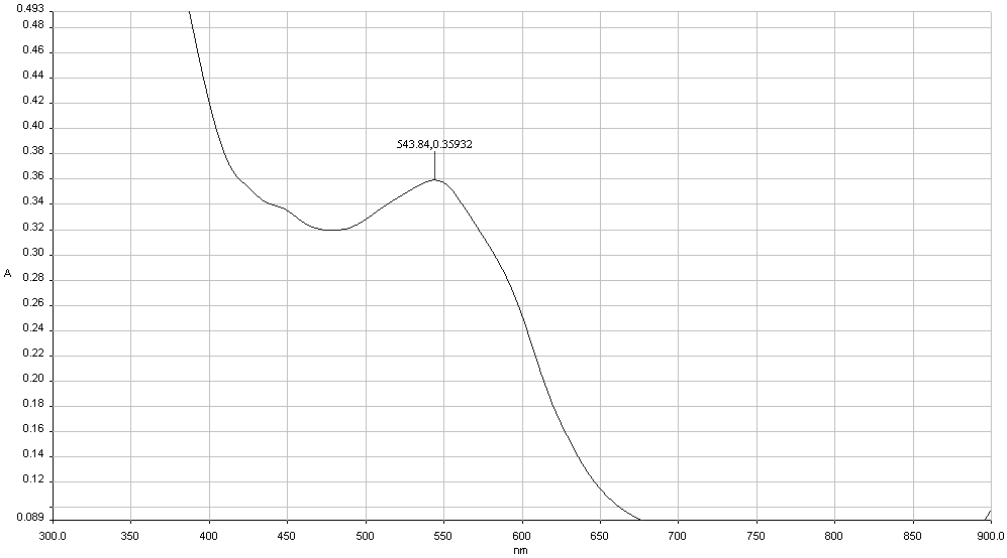


Fig. 1. Absorption spectra of black raspberry extracts in ethanol (1 ml concentrated extract + 3 ml ethanol)

A higher intensity of chemiluminescent light was observed after the addition of vitamin C than with the *R. niveus* extract, demonstrating the scavenging capacity of these antioxidants against the ·OH radical.

The RSC for *R. niveus* extract was 59.98±3.78, and 70.56±1.26 for vitamin C (where 3.78 and 1.26 represent the standard deviation of the triplicate measurements made for each respective case). **Figure 5** shows the scavenging activity of the *R. niveus* extract and its comparison with vitamin C in graphical form.

Figure 6 shows the reaction of black raspberry ethanolic extract with the galvinoxyl radical. It can be seen that the absorption spectrum of this radical has two major absorption points: one at ~380 nm and another at ~430 nm. Furthermore, it is evident that, with the addition of *R. niveus* extract, the absorption intensity at ~380 nm increases, while that at ~430 nm decreases.

On the other hand, **figure 7** shows the course of the reaction of *R. niveus* extract with the galvinoxyl radical using a fluorescence spectrum (with excitation wavelength at 350). It can be seen that the intensity of the excitation light from galvinoxyl radical decreases after addition of black raspberry extract.

Figure 8 shows the reaction of black raspberry extract with singlet oxygen in the presence of Rose Bengal (a type II photosensitizer). The reaction shows that, after irradiation for 2 hours, the wavelength decreases in intensity, with a shift from 544 nm to 549 nm. A similar situation occurs after irradiating for an additional hour, as the wavelength remains at ~549 nm with a corresponding decrease in intensity.

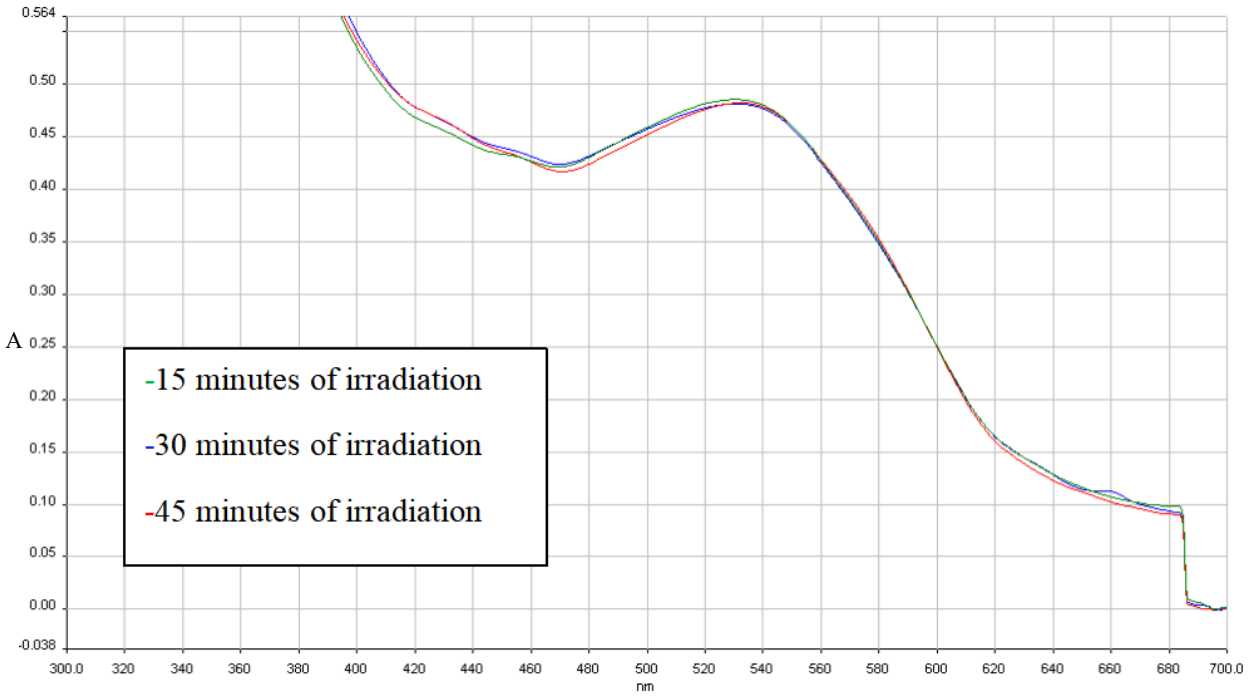
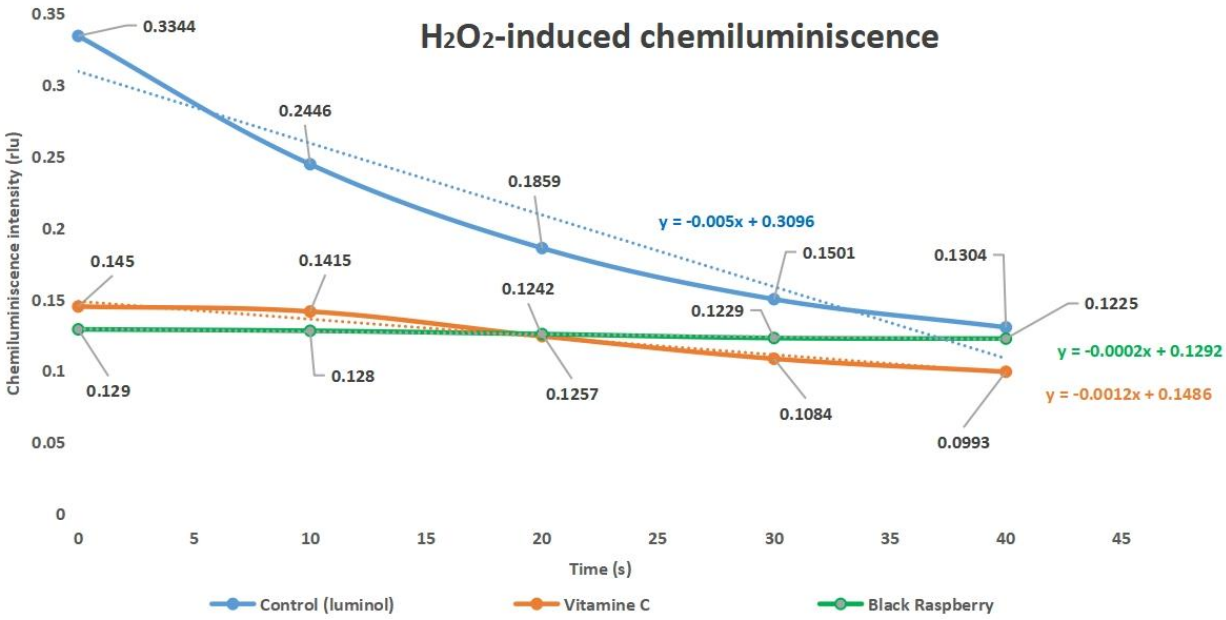
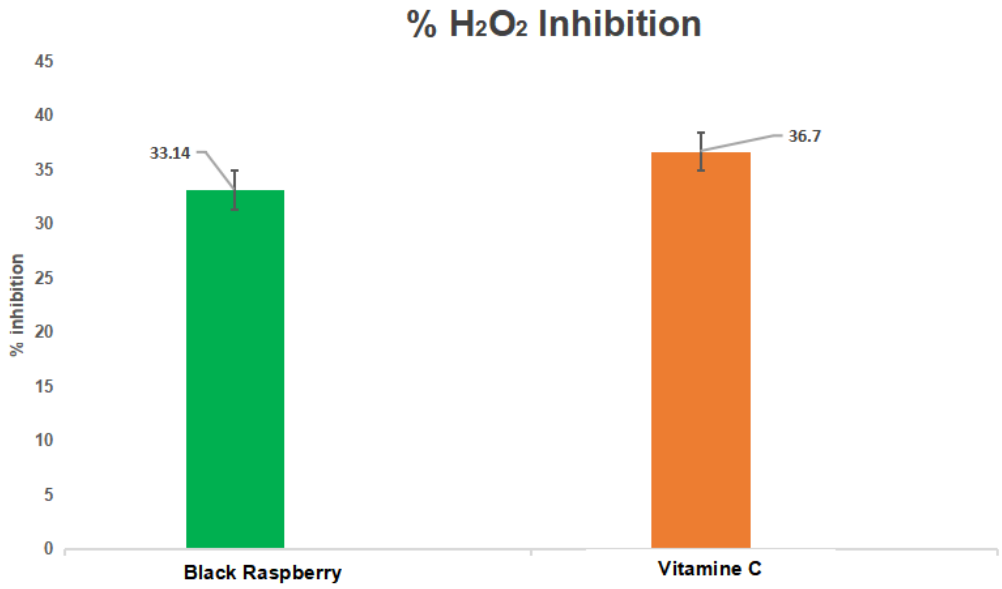


Fig. 2. Photostability tests of raspberry extract under irradiation with a solar simulator

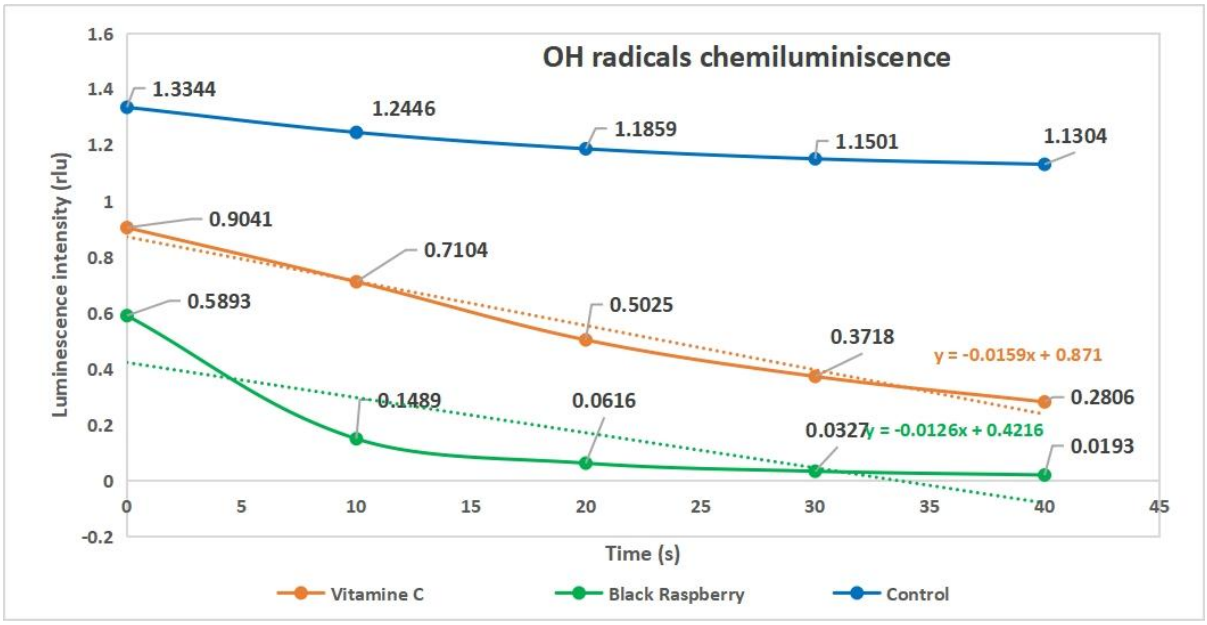


(a)

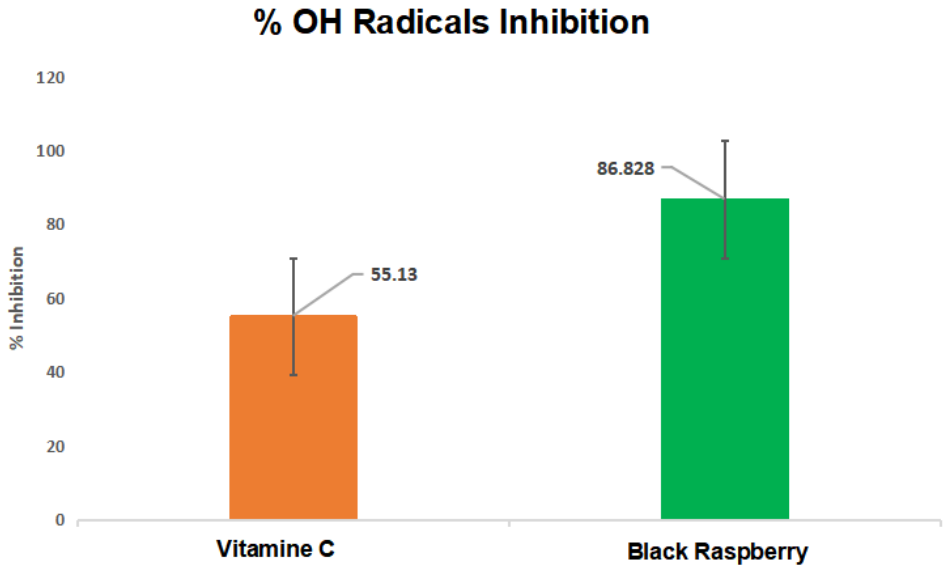


(b)

Fig. 3. H_2O_2-induced chemiluminescence assays



(a)



(b)

Fig. 4. Ferrous iron-induced chemiluminiscence

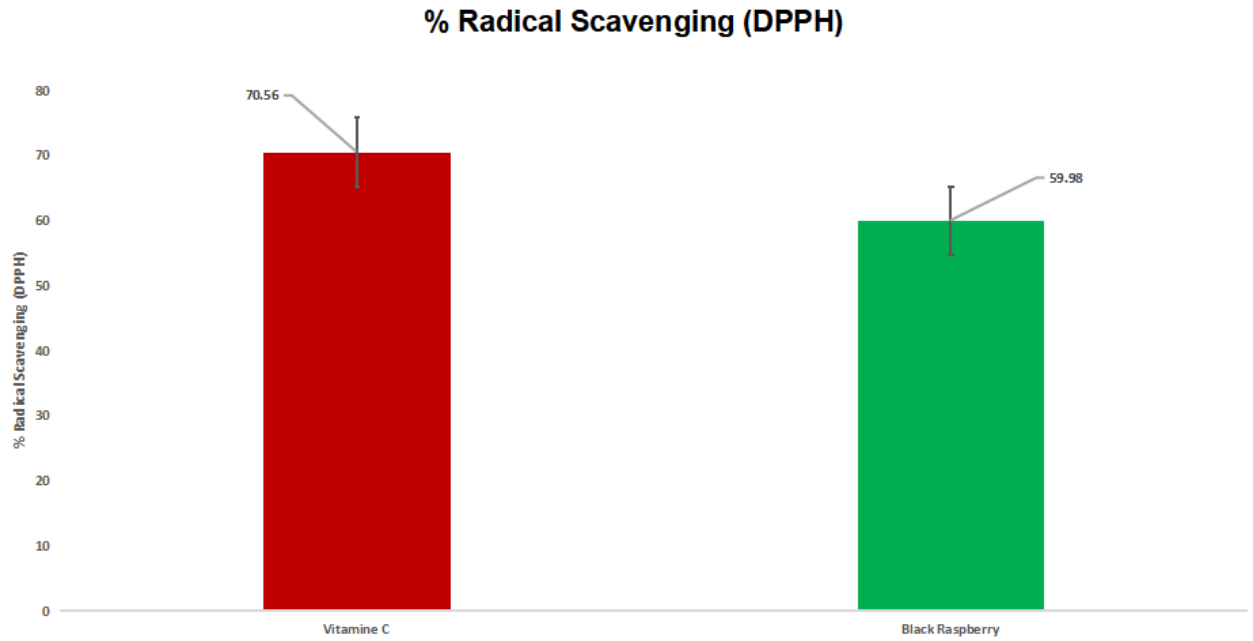


Fig. 5. Radical scavenging (RSC) from black raspberry extract and vitamin C

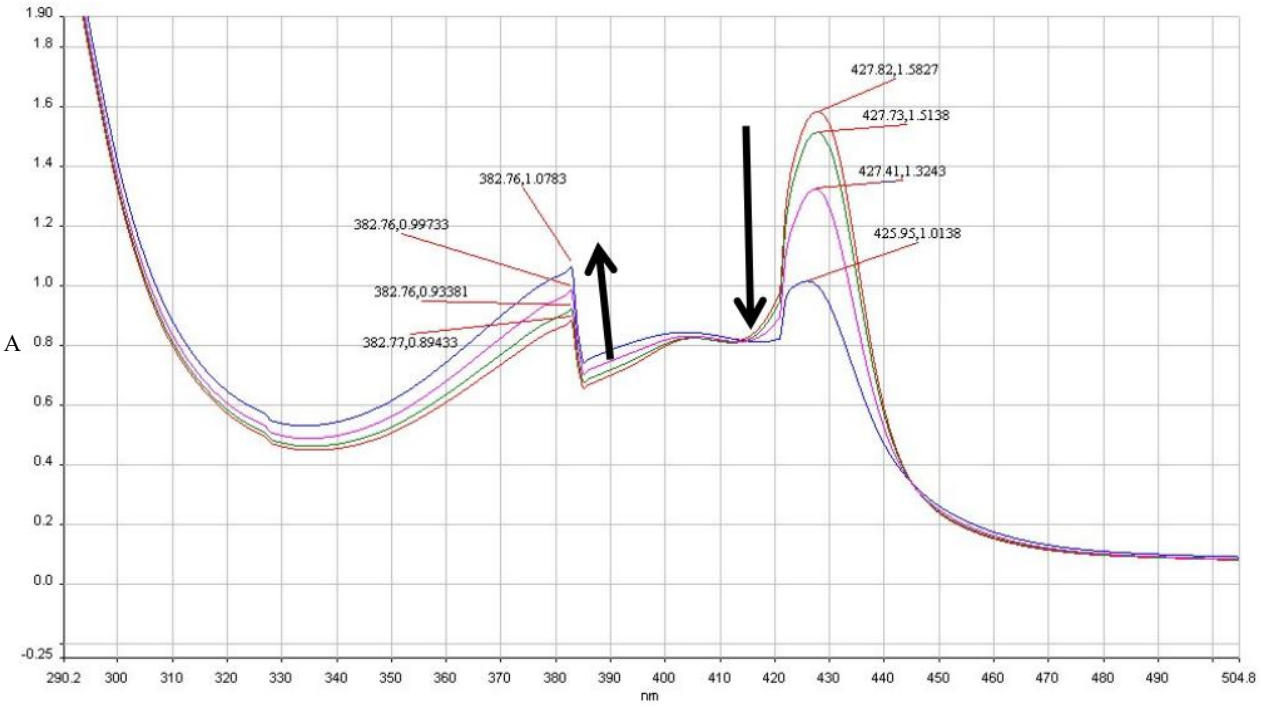


Fig. 6. Reaction of black raspberry extract with galvinoxyl radical followed by UV-VIS spectroscopy

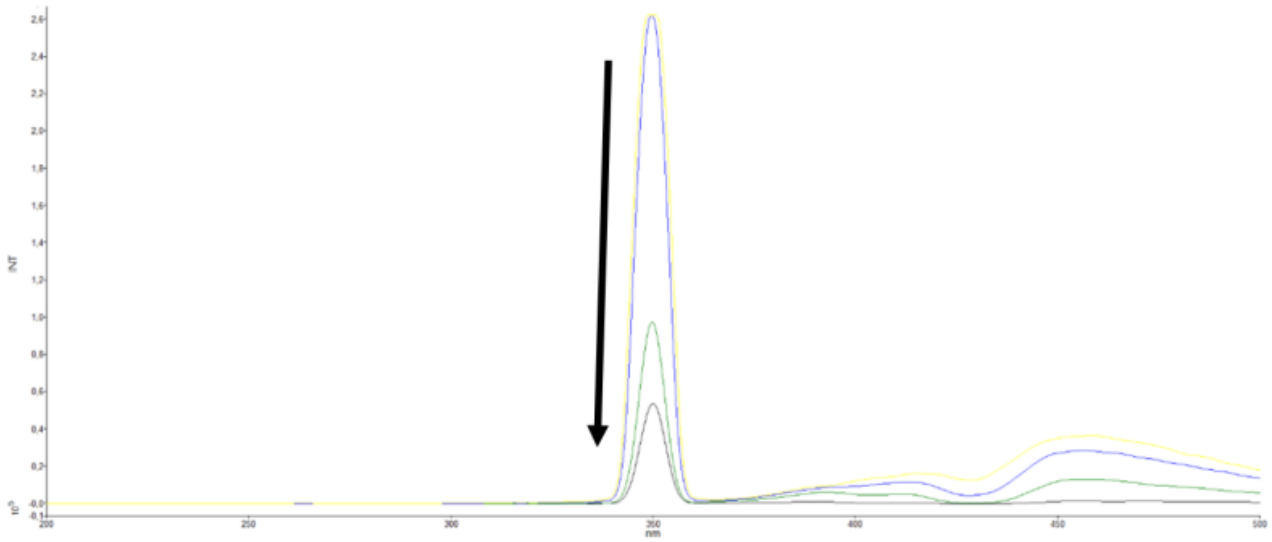


Fig. 7. Reaction of black raspberry extract with galvinoxyl radical using excitation band

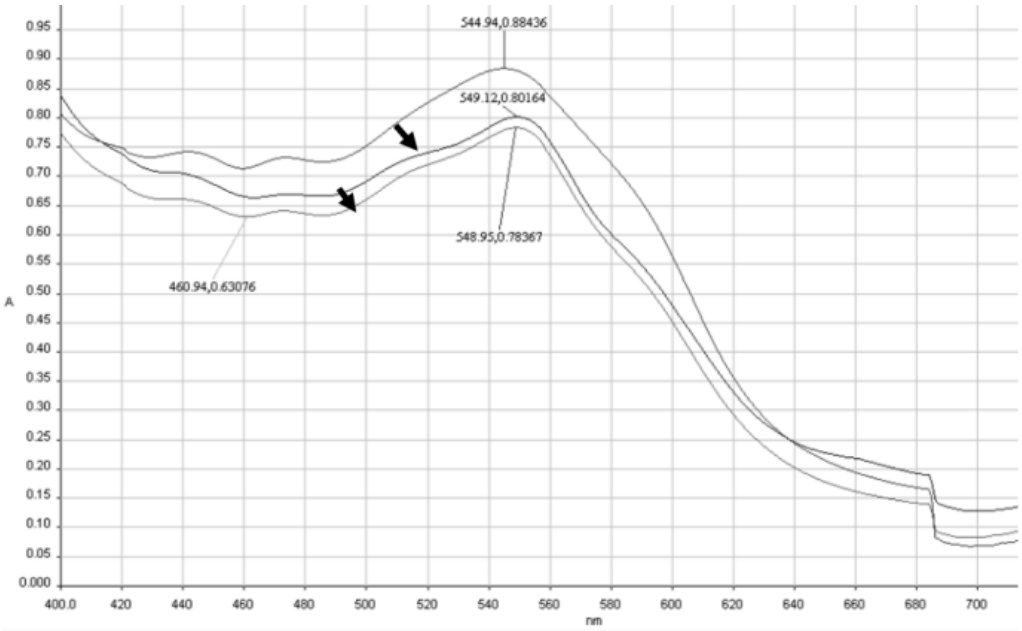
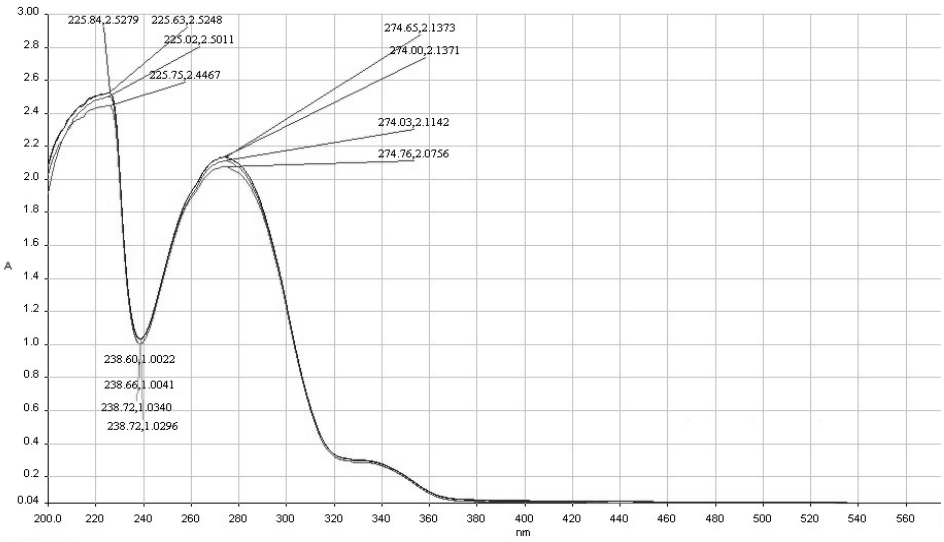


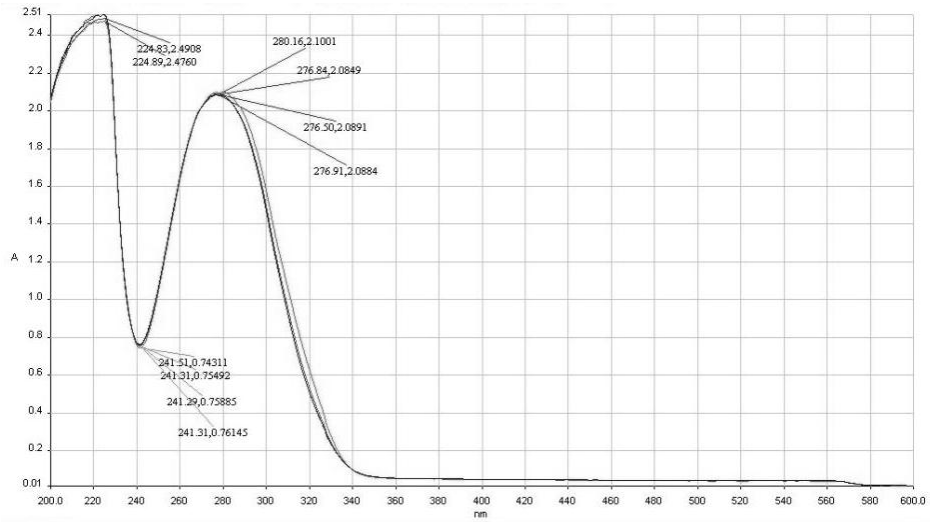
Fig. 8. Decrease in the maximum absorbance of black raspberry extract in the presence of Rose Bengal (~2x10^{-4} M) after irradiation for 2 hours + 1 hour with white light.

It should be noted that the results presented in **figure 2** offer compelling evidence for the photostability of black raspberry extract (*R. niveus*), as evidenced by the minimal degradation observed at the maximum wavelength following irradiation with solar simulator, indicating that there is not photodegration processes during irradition (Ahdmad et al., 2016), suggesting the stability capacity of *R. niveus* extract under solar irradiation.

As illustrated in **figures 9.a** and **9.b**, the photostability behavior of common sunscreen filters under visible radiation (i.e., solar simulator) is evident. The filters in question include Neo Heliopan type Ma (menthyl anthranilate) and Neo Heliopan type OS (Octisalate, 2-Ethylhexyl salicylate).



(a)



(b)

Fig. 9. Photostability under Visible radiation of (a) Neoheliopan Type Ma and (b) Neoheliopan Type OS

The response of each constituent, dissolved in dichloromethane due to the lack of solubility of these compounds in water-ethanolic mixtures, to visible light was minimal following irradiation, as demonstrated by negligible shifts in the maximum absorption.

A comparison of the results obtained with the irradiation of black raspberry extract reveals that the polyphenols present in *R. niveus* fruit exhibit a photostability against solar simulator radiation analogous to that observed for sunscreen filters (**Figure 9**).

The latter suggests the potential of ethanolic *R. niveus* extract to provide photostability *in vitro*.

On the other hand, the antioxidant potential of the *R. niveus* ethanolic extract was evidenced by its capacity to diminish the presence and intensity of reactive oxygen species, specifically H_2O_2 and OH radicals. In reactions against these radicals, a comparison with vitamin C reveals discrepancies in their efficacy. The *R. niveus* extract demonstrated a lower percentage of inhibition for H_2O_2 (33.14%) in comparison to vitamin C (36.7%), as illustrated in Figure 3.b. However, while the

capacity to neutralize H_2O_2 is reduced, *R. niveus* has been observed to have a greater potential to inhibit ·OH radicals, as shown in figure 4.b **(**86.82% of inhibition for *R. niveus* and 55.13% of inhibition for vitamin C**).** This phenomenon can be attributed to the observation that the chemiluminescent light intensity in the presence of the extract is lower in comparison to the reaction with vitamin C (**Figure 3.a**). These findings suggest that the phenolic compounds present in the *R. niveus* fruit have an exclusive affinity for highly reactive radicals, which may be fundamental to preventing damage caused by oxidative stress (Speer et al., 2020).

In addition, the scavenging capacity of *R. niveus* extract against the galvinoxyl radical is significant, since the absorbance of the radical decreases at 430 nm and increases at 370 nm. This suggests that a transformation of a more reactive radical (galvinoxyl) to a less reactive one does indeed occur, promoted by the ·OH functional groups commonly found in phenolic compounds, which contribute to the antioxidant potential of *R. niveus* extract. Additionally, assays with the DPPH radical show that the RSC was 59.98%, which, although lower than the 70.56% for vitamin C, indicates significant potential for free radical scavenging (Bobinaitė et al., 2012; Gulcin and Alwasel, 2023).

The significance of the previous results is evident in **Table 1**. The differences in the results can be explained by analyzing the p-value; $p<0.05$ indicates a statistically significant difference in the experimental results. In this case, there was no significant difference in the H_2O_2 assay. Thus, despite the different percentages of H_2O_2 inhibition (33.14% for black raspberry and 36.7% for vitamin C), vitamin C and black raspberry exhibit similar behavior in neutralizing peroxides. However, according to the slopes of the equations in **figure 3a**, vitamin C has a more stable capacity to neutralize peroxides over time. Black raspberry, on the other hand, exhibits faster inhibition at the initial stage of the chemiluminescent reaction. This suggests that the inhibitory effect of vitamin C is more sustained than that of black raspberries in neutralizing. In other words, black raspberries tend to have a faster initial effect on neutralizing peroxides than vitamin C, although the neutralization capacity of black raspberry decays over time. However, significant differences were observed in the OH radical assays, confirming that black raspberry extract has a stronger capacity to scavenge OH free radicals *in vitro*. The calculated difference is statistically significant: 86.828% for black raspberry versus 55.13% for vitamin C. Therefore, black raspberry can be assumed to be a potent antioxidant capable of quickly and effectively neutralizing a large number of OH radicals (see the equation of lines in **figure 4.a**). However, the DPPH assay results indicated that vitamin C (70.56% of inhibition) has stronger scavenging activity against DPPH radicals than black raspberry extract (59.98% of inhibition).

Table 1. Unpaired t-test results analysis

	T-test	
	T value	**P value**
H_2O_2 assay	0.3090	0.7652
OH assay	2.8350	0.0220
DPPH assay	6.2586	0.0033

These differences can be explained on a structural basis (Kim and Lee, 2004; Yamauchi et al., 2024). While discussing these contradictory results is beyond the scope of this research, they can be attributed to the specificity of polyphenols and vitamin C against DPPH molecules and free OH radicals. This assumes greater specificity of vitamin C and DPPH radicals than the polyphenolic components of black raspberry fruit.

Thus, it is necessary to consider the reaction of black raspberry extract with the galvinoxyl radical. Fluorescence studies using excitation bands (in this case at 350 nm) indicate that the reaction follows both a scavenging mechanism and a quenching mechanism. This is due to the fact that the excitation band of the galvinoxyl radical, as detected by the instrument, decreases following the addition of the ethanolic extract of *R. niveus*. In this regard, it is proposed that the black raspberry extract may function as a quencher of the excited states of free radicals. These mechanisms may be analogous to those previously suggested in studies related to free radical reactions and quenching (Khudyakov, 2023; León and Tovar, 2025). It is important to note that the excitation spectrum of the galvinoxyl radical was selected because it is a radical species whose fluorescent emission is not detectable with conventional equipment, although fluorescent species derived from this radical, such as the galvinoxylate anion, may exist (Grilj et al., 2012).

Conversely, the reaction of the ethanolic extract with singlet oxygen demonstrate that *R. niveus* is susceptible to the presence of this free radical, as evidenced by a decrease in the maximum wavelength after each measurement. While this behavior may compromise the integrity of the ethanolic extract, it may be advantageous for biological applications. The presence of compounds in black raspberry fruit that react with $^1O_2$ suggests that this ROS may dissipate over time, and protect from damage that is usually attributed to this type of ROS (Tournaire et al., 1993; De Rosso et al., 2008).

A comparison of the results of this study with those of previous research reveals that the anthocyanin content was lower than the values reported in previous studies of *Rubus niveus* fruit. This study obtained values of 17.09 mg/100 g (León and Soledad, 2020) and 5.63 mg/100 g (Badhani et al., 2015), whereas the previous study obtained a value of 48.19 mg/100 g of fresh weight. These differences can be attributed to different solvents, calculated dilution factors, and extraction conditions. The ripeness of the selected fruits, as well as picking and conservation conditions (e.g., elevation of the cultivation area, climate, and storage), may also influence the reported anthocyanin content in *R. niveus* fruit (Maro et al., 2013).

Additionally, it is well established that *R. niveus* has a higher anthocyanin content and radical scavenging capacity than the wild yellow raspberry, *R. ellipticus*. For instance, Ahmad et al. (2015) demonstrated that the DPPH radical scavenging capacity of cultivated Himalayan *R. niveus* berries was 68.30%, while the capacity of wild *R. ellipticus* berries was 45.97%. However, inhibition of *R. ulmifolius* berries was higher (80.28%), and the scavenging potential of *R. niveus* was higher than the vitamin C standard (53.73%). The differences between this study and our research can be explained by the different cultivation and extraction procedures; the latter study used methanolic extracts. In our study, we employed ethanolic extracts of *R. niveus*, and the fruits were collected in an area at a lower altitude than the Himalayan mountains. This difference in altitude may significantly affect the content of polyphenolic components in the fruits (Maro et al., 2013).

A comparison of the properties of *R. niveus* and the commercially available black

raspberry, *Rubus occidentalis*, reveals similar levels of anthocyanin compounds in both species. Total amounts of these molecules have been reported to range from 20 to 216 mg/100 g of fresh fruit and 1,770 mg/100 g of freeze-dried fruit. Once again, it is evident that cultivation, storage, and extraction conditions significantly impact the availability of phenolic compounds (Maro et al., 2013). In addition, different RSC_{30} values were observed in black raspberry varieties (*R. occidentalis*) grown in Lithuania, ranging from 25 to 80%, depending on the variety (Viškelis et al., 2010). Despite the different locations where the fruit was grown and harvested, as well as the different extraction conditions used in each case, these tests were carried out with methanolic extracts and obtained values close to those reported in this study (59.98% RSC_{30} for *R. niveus*).

Additionally, ethanol extracts of Korean black raspberry (*R. coreanus*) exhibited RSC activity, demonstrating 60-80% inhibition. These differences are primarily due to the concentration of ethanol (EtOH) used: the extract with 100% EtOH exhibited the lowest activity, while the extract with 50% EtOH exhibited the highest activity. These results show that the type of solvent is closely related to the *in vitro* antioxidant activity of natural black raspberry extracts (Kim et al., 2014).

This study reports the values of wild *R. niveus* fruit and compares them to those of other berry sources. This comparison highlights the fruit's significant antioxidant potential, particularly its capacity to scavenge radicals like DPPH. However, this capacity is slightly lower than that of vitamin C in this specific assay (59.98% vs. 70.56%). Furthermore, despite the differences in methodology and cultivation conditions, the anthocyanin content is within the expected range and comparable to that of other blackberry sources. For example, *R. occidentalis* and *R. coreanus* have reported anthocyanin content values of 6.7, 165, and 312.2 mg/100 g of fresh weight, depending on the genotype of the fruit (Ku and Mun, 2008).

In summary, the ethanolic extract of *Rubus niveus* fruit consists primarily of anthocyanin molecules, as demonstrated by UV-Vis spectra and the differential pH method (which can only detect monomeric anthocyanins). These flavonoid compounds are well known for their high antioxidant capacity (especially against OH radicals, that was significantly higher than vitamin C), suggesting that the scavenging activity demonstrated in the results of this study is mainly due to this class of molecules, although other compounds present in black raspberries, such as catechins or tannins, may also play an important role in photostability and antioxidant potential (Ahmad et al., 2015).

Conclusions

The ethanolic extract of black raspberry fruit (*Rubus niveus*) is a source of flavonoid compounds, especially anthocyanins, as demonstrated by UV-Vis spectroscopy and the differential pH method. This property is crucial for its high antioxidant capacity against reactive oxygen species (ROS), including H_2O_2, OH, and $^1O_2$. This behavior is complemented by the scavenging and quenching potential demonstrated by the extract against galvinoxyl and DPPH radicals. In addition, the results provide a comprehensive explanation for the photostability of the *R. niveus* extract under solar light, after comparison with spectral changes of common sunscreen filters. Future research will be conducted in our laboratory to determine the different types of flavonoids and other natural components present in *R. niveus* fruit, which might be of photochemical and photobiological interest.

Despite these findings, it is important to acknowledge the limitations of this research. The extraction solvent, dilution factors, and environmental conditions (e.g., altitude,

climate, fruit ripeness, and storage conditions) in which the fruit was collected and processed may impact the specific anthocyanin content obtained, as well as the observed scavenging and quenching activities. These factors may also affect the generalization of quantitative comparisons with other studies. Additionally, although anthocyanins are recognized as significant contributors, providing a comprehensive description of all the flavonoid types and other bioactive substances present in the extract was beyond the scope of this study.

Finally, research on formulation studies, bioavailability, mechanistic pharmacology, dermatological models, and stability in pharmaceutically relevant matrices would be desirable. A deeper comparison with established photoprotective actives would provide mechanistic insights at the chemical, cellular, and molecular levels. Therefore, further *in vitro* and *in vivo* research is essential to corroborating the antioxidant and photoprotective mechanisms of *R. niveus* for biological and product applications.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Aguilera-Otíz M, Del Carmen Reza-Vargas M, Chew-Madinaveita RG, Meza-Velázquez JA. (2011). PROPIEDADES FUNCIONALES DE LAS ANTOCIANINAS. BIOtecnia, 13(2), 16. doi: 10.18633/bt.v13i2.81
2. Ahmad I, Ahmed S, Anwar Z, Sheraz MA, Sikorski M. (2016). Photostability and photostabilization of drugs and drug products. International Journal of Photoenergy, 2016, 1–19. doi: 10.1155/2016/8135608
3. Ahmad M, Masood S, Sultana S, Hadda TB, Bader A, Zafar M. (2015). Report: antioxidant and nutraceutical value of wild medicinal Rubus berries. PubMed, 28(1), 241–247. doi: pubmed.ncbi.nlm.nih.gov/25553701
4. Bachheti A, Deepti N, Bachheti RK, Singh A, Zebeaman M, Hunde Y, Husen A. (2023). Bioactive constituents and health promoting compounds of underutilized fruits of the northern Himalayas of India: a review. Food Production Processing and Nutrition, 5(1). doi: 10.1186/s43014-023-00140-5
5. Badhani A, Rawat S, Bhatt ID, Rawal RS. (2015). Variation in Chemical Constituents and Antioxidant Activity in Yellow Himalayan (R ubus ellipticus Smith) and Hill Raspberry (R ubus niveus Thunb.). Journal of Food Biochemistry, 39(6), 663–672. doi: 10.1111/jfbc.12172
6. Bobinaitė R, Viškelis P, Venskutonis, PR (2011). Variation of total phenolics, anthocyanins, ellagic acid and radical scavenging capacity in various raspberry

(Rubus spp.) cultivars. Food Chemistry, 132(3), 1495–1501.
doi: 10.1016/j.foodchem.2011.11.137

7. Chiluisa-Utreras V, Vaca I, Chicaiza O, Peñaherrera S. (2017). Analysis of the expression of β-actin, RuANS and RuMYB10 genes involved in the biosynthesis of anthocyanin using RT-qPCR in Rubus niveus in the Rumiñahui Canton. Journal of Berry Research, 7(3), 195–201. doi: 10.3233/jbr-170156
8. Da Silva Mendonça J, De Cássia Avellaneda Guimarães R, Zorgetto-Pinheiro VA, Di Pietro Fernandes C, Marcelino G, Bogo D, De Cássia Freitas K, Hiane PA, De Pádua Melo ES, Vilela MLB, Nascimento VaD. (2022). Natural Antioxidant Evaluation: A Review of Detection Methods. Molecules, 27(11), 3563. doi: 10.3390/molecules27113563
9. Dangles O, Fenger J. (2018). The chemical reactivity of anthocyanins and its consequences in food science and nutrition. Molecules, 23(8), 1970. doi: 10.3390/molecules23081970
10. De Rosso VV, Vieyra FEM, Mercadante AZ, Borsarelli CD. (2008). Singlet oxygen quenching by anthocyanin's flavylium cations. Free Radical Research, 42(10), 885–891.
doi: 10.1080/10715760802506349
11. Grilj J, Zonca C, Daku LML, Vauthey E. (2012). Photophysics of the galvinoxyl free radical revisited. Physical Chemistry Chemical Physics, 14(18), 6352. doi: 10.1039/c2cp23577c
12. Gulcin İ, Alwasel SH. (2023). DPPH radical scavenging assay. Processes, 11(8), 2248. doi: 10.3390/pr11082248
13. a. Hertog MGL, Hollman PCH, Katan MB, Kromhout D. (1993). Intake of potentially anticarcinogenic flavonoids and their determinants in adults in the Netherlands. Nutrition and Cancer, 20(1), 21–29. doi: 10.1080/01635589309514267
14. b. Hertog M, Feskens E, Kromhout D, Hertog M, Hollman P, Hertog M, Katan M. (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. The Lancet, 342(8878), 1007–1011.
doi: 10.1016/0140-6736(93)92876-u
15. Khoo HE, Azlan A, Tang ST, Lim SM. (2017). Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food & Nutrition Research, 61(1), 1361779.
doi: 10.1080/16546628.2017.1361779
16. Khudyakov IV(2023). Electronically excited states of free radicals. Physchem, 3(3), 332–341.
doi: 10.3390/physchem3030023
17. Kim D, Lee CY. (2004). Comprehensive Study on Vitamin C Equivalent Antioxidant Capacity (VCEAC) of Various Polyphenolics in Scavenging a Free Radical and its Structural Relationship. Critical Reviews in Food Science and Nutrition, 44(4), 253–273.
doi: 10.1080/10408690490464960
18. Kim LS, Youn SH, Kim JY. (2014). Comparative Study on Antioxidant Effects of Extracts from Rubus coreanus and Rubus occidentalis. Journal of the Korean Society of Food Science and Nutrition, 43(9), 1357–1362.
doi: 10.3746/jkfn.2014.43.9.1357
19. Ku CS, Mun SP (2008). Optimization of the extraction of anthocyanin from Bokbunja (Rubus coreanus Miq.) marc produced during traditional wine processing and characterization of the extracts. Bioresource technology, 99(17), 8325–8330. doi: 10.1016/j.biortech.2008.03.013
20. Lee J, Durst RW, Wrolstad RE, Eisele T, Giusti MM, Hach J, Hofsommer H, Koswig S, Krueger DA, Kupina S, Martin SK, Martinsen BK, Miller TC, Paquette F, Ryabkova A, Skrede G, Trenn U,

Wightman JD. (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. Journal of AOAC International, 88(5), 1269–1278. doi: 10.1093/jaoac/88.5.1269

21. León M, Soledad B (2020). Estudio del perfil de antocianinas en Rubus Niveus utilizando cromatografía líquida (HPLC) y pH diferencial. Rev EDUCAB, 11:147-56. doi: 20.500.12032/121468
22. León M, Tovar FV. Radical scavenging activity of polyphenolic contained in the Black Raspberries. An assay on absorption and emissión espectra of galvinoxyl radical (2025)., in Proceedings of the 29th International Electronic Conference on Synthetic Organic Chemistry, 14–28, MDPI: Basel, Switzerland, doi:10.3390/ecsoc-29-26909
23. Li J, Du L, He Y, Yang L, Li Y, Wang Y, Chai X, Zhu Y, Gao X. (2015). Chemical Constituents and Biological Activities of Plants from the Genus Rubus. Chemistry & Biodiversity, 12(12), 1809–1847. doi: 10.1002/cbdv.201400307
24. Maro LaC, Pio R, Guedes MNS, De Abreu CMP, Curi PN. (2013). Bioactive compounds, antioxidant activity and mineral composition of fruits of raspberry cultivars grown in subtropical areas in Brazil. Fruits, 68(3), 209–217. doi: 10.1051/fruits/2013068
25. Muniyandi K, George E, Sathyanarayanan S, George BP, Abrahamse H, Thamburaj S, Thangaraj P. (2019). Phenolics, tannins, flavonoids and anthocyanins contents influenced antioxidant and anticancer activities of Rubus fruits from Western Ghats, India. Food Science and Human Wellness, 8(1), 73–81. doi: 10.1016/j.fshw.2019.03.005
26. Nesello LAN, Beleza MLML, Mariot M, Mariano LNB, De Souza P, Campos A, Cechinel-Filho V, Andrade SF, Da Silva LM. (2017). Gastroprotective Value of Berries: Evidences from Methanolic Extracts of Morus nigra and Rubus niveus Fruits. Gastroenterology Research and Practice, 2017, 1–8. doi: 10.1155/2017/7089697
27. Pancholi B, Rana AC. (2020). Traditional Uses, Phytochemistry and Pharmacological Aspects of Rubus niveus thumb Plant – A Review. The Journal of Phytopharmacology, 9(6), 438–444. doi: 10.31254/phyto.2020.9610
28. Parimelazh T, George B, Saravanan S, Chandran R. (2013). Anti-inflammatory, Analgesic and Antipyretic Properties of Rubus niveus Thunb. Root Acetone Extract. Pharmacologia, 4(3), 228–235. doi: 10.5567/pharmacologia.2013.228.235
29. Silva VO, Freitas AA, Maçanita AL, Quina FH. (2016). Chemistry and photochemistry of natural plant pigments: the anthocyanins. Journal of Physical Organic Chemistry, 29(11), 594–599. doi: 10.1002/poc.3534
30. Speer H, D'Cunha NM, Alexopoulos NI, McKune AJ, Naumovski N. (2020). Anthocyanins and Human Health—A focus on oxidative stress, inflammation and disease. Antioxidants, 9(5), 366. doi: 10.3390/antiox9050366
31. Tournaire C, Croux S, Maurette M, Beck I, Hocquaux M, Braun AM, Oliveros E. (1993). Antioxidant activity of flavonoids: Efficiency of singlet oxygen (1Δg) quenching. Journal of Photochemistry and Photobiology B Biology, 19(3), 205–215. doi: 10.1016/1011-1344(93)87086-3
32. Vargas F, Díaz Y, Carbonell K. (2004). Antioxidant and scavenging activity of emodin, Aloe-Emodin, and rhein on Free-Radical and reactive oxygen species.

Pharmaceutical Biology, 42(4–5), 342–348. doi: 10.1080/13880200490519613

33. Viskelis P, Rubinskiene M, Bobinaite R, Dambrauskiene E. (2010). Bioactive compounds and antioxidant activity of small fruits in Lithuania. Journal of Food Agriculture & Environment, 8, 259–263. doi: cabdirect/abstract/20113031189
34. Yamauchi M, Kitamura Y, Nagano H, Kawatsu J, Gotoh H. (2024). DPPH Measurements and Structure—Activity relationship studies on the antioxidant capacity of phenols. Antioxidants, 13(3), 309. doi: 10.3390/antiox13030309
35. Zvereva MV, Zhmurova AV. (2023). The use of a chemiluminescence in the assessment of the nanomaterials antioxidant activity. Biophysical Reviews, 15(5), 963–969. doi: 10.1007/s12551-023-01148-4

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