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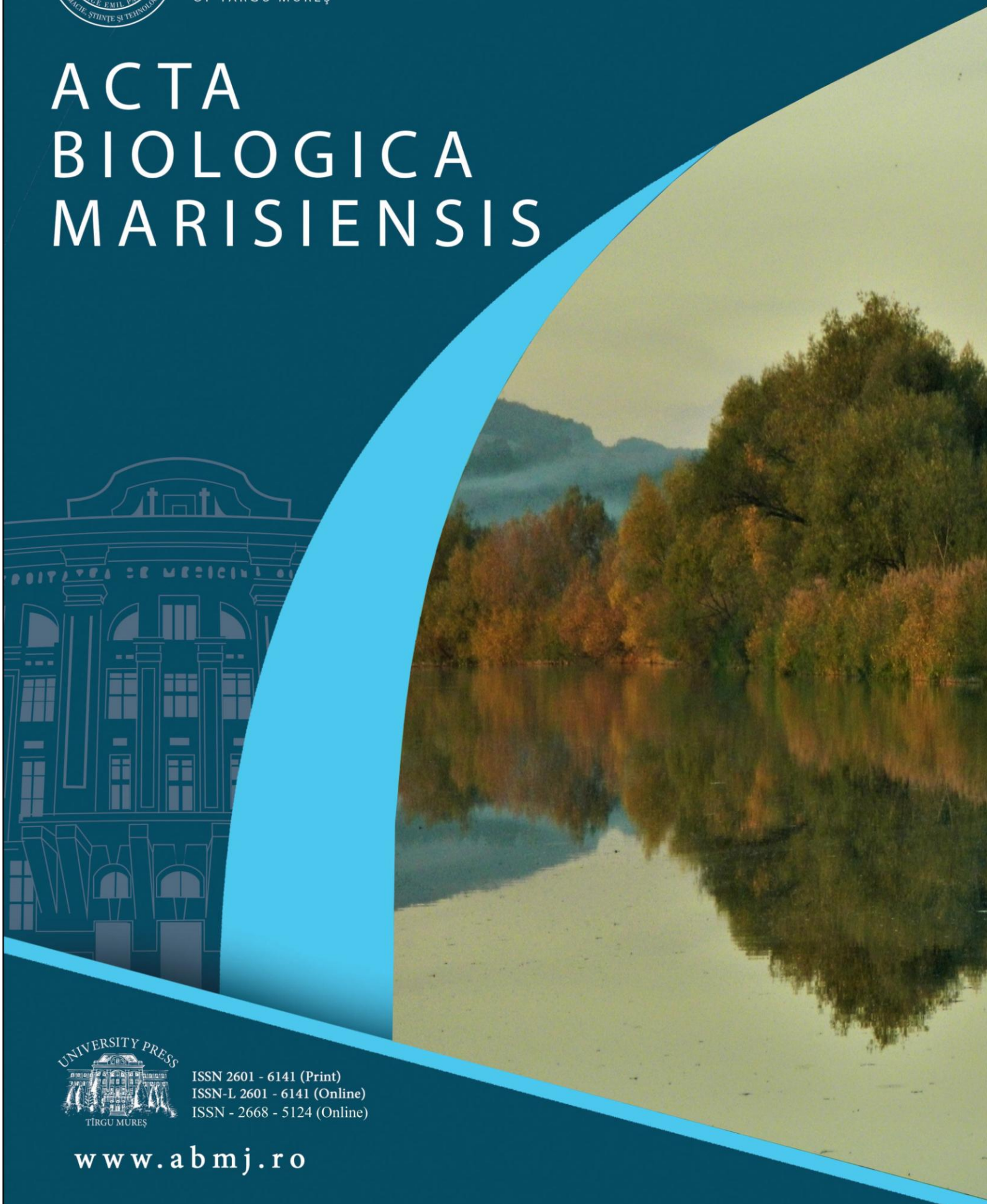

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MORPHOLOGICAL AND ANATOMICAL PROFILE OF *CASSIA OCCIDENTALIS* (L.) SPECIES GROWN IN THE REPUBLIC OF MOLDOVA

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Abstract: The current research deals with the morpho-anatomical characteristics of *Cassia occidentalis* (L.) (Coffee senna plant), grown in the steppe climate conditions of the Republic of Moldova. This study was performed according to investigated organ in vegetative periods of 2019-2021 years. Morphological and anatomical indices of organs with diagnostic role in identifying species and those with adaptive potential to the action of unfavorable growth factors, have been elucidated. These indices were evaluated in the light of data already reported by other researchers. This morpho-anatomical research has revealed new aspects to support some anatomical features already outlined, but less confirmed in the studies of the last decades and will serve as landmarks for a real clarity of the integral microstructural picture of the *C. occidentalis* species.

Keywords: *Cassia occidentalis*, *Senna occidentalis*, morphology, anatomy, Republic of Moldova

1. Introduction

Morphological and taxonomic data of *Cassia occidentalis* (L.) (synonym of *Senna occidentalis* (L.) Link) species is very confusing in the evaluated bibliography, sometimes even contradictory (Kumar, 2009; Begum et al., 2014; Fonkou et al., 2018). We agree, as taxonomic monographs are now considered incomplete without epidermal micromorphology (Rejdali, 1991) in particular, and in general without microscopic characteristics. We believe that microscopic studies can provide clarity in this aspect. In this regard, we refer to recent microscopic studies on some species of g. *Cassia* in general (Sahai, 2001; Ogundipe et al., 2009) and *S. occidentalis* in particular (Nassar et al., 2013; Begum et al., 2014; Doty et al., 2020). This species is known in English as Coffee senna, Coffeeweed, Piss-a-bed, Mogdad coffee, Negro-coffee, Senna coffee, Stephanie coffee, Sinkingweed or Styptic weed, but the most used English common name is Coffee senna (Allsopp, 1996). The current investigation concerns the morphology and anatomy of Coffee senna plants, grown in the climate conditions of the Republic of Moldova, situated in southeastern Europe, and characterized by a predominantly temperate steppe climate. The

species was introduced into Collection of Medicinal and Aromatic Plans of the Institute of Genetics, Physiology and Plant Protection (IGPPP) in 2017 and has been propagated from its own seeds in the following period. The morpho-anatomical structure of plant organs was performed in the mass flowering period of the plant during the growing season of 2020-2021 years.

The Coffee senna plants contain valuable chemical constituents like: sennosides and anthraquinones (pods); dianthronic heterosides (leaves); apigenin (pericarp); emodol (roots); betasitosterol (flowers); volatile oils (leaves, roots, seeds) used to combat illness and support the body's own defense to regain good health (Khare, 2004; Yadav et al., 2010; Vijayalakshmi et al., 2013; Ngombe et al., 2019). These metabolites are responsible for numerous pharmacological properties: antidiabetic, antimicrobial, antioxidant, anti-inflammatory, anticarcinogenic and antimalarial. The Coffee senna plant also possesses purgative, tonic, febrifugal, expectorant, and diuretic activities (Singh et al., 2016). However, one of the best solutions for securing the necessary raw materials for phytopharmaceuticals is to cultivate this species on a scientific basis.

Obviously, the morpho-anatomical data on the species *S. occidentalis* cultivated in the climatic conditions of the Republic of Moldova are of particular interest both for determining the potential structural adaptability to the action of unfavorable environmental factors and from the point of view of the possibility of cultivation and exploitation of this species for medicinal purposes.

2. Materials and methods

The anatomical structure of the plant organs was performed during the mass flowering period of the plant in the Collection of Medicinal and Aromatic Plants of IGPhPP during the growing season of 2020-2021 years. The microscopic study was carried out at the Department of Pharmacognosy and Pharmaceutical Botany of Nicolae Testemițanu State University of Medicine and Pharmacy.

The studied organs were: main root and main stem (cross-sections and superficial preparations of rhizodermis and epidermis), leaf (cross-sections through rachis and through leaflets in median region, superficial preparations of adaxial and abaxial epidermises), flower (superficial preparations of sepals and petals), fruit (cross-sections through pericarp and superficial preparations of outer and inner epidermises), seed (cross-section through seed coat and cotyledons). The micropreparations was carried out by applying classical techniques to obtain cross-sections and surface preparations from fresh, dried and clarified botanical material in hydrochloride solution. Selective staining reagents were applied to identify the chemical nature of some structures according to methodical recommendations (Nistreanu and Calalb, 2016; Calalb and Nistreanu, 2021).

Test for anthraquinones

2 ml of aqueous extract was boiled with 5 ml of 10% HCl for 3 minutes followed by further addition of 5 drops of 10% ammonia. A rose-reddish coloration indicates a positive result.

Test for lipids

A few drops of Sudan III solution were applied to the sections for 2-4 hours. The lipid globules are stained in red.

Test for cell wall

A few drops of Zn-Cl-I solution were applied to the sections. The lignified cell walls are colored in dark orange and cellulose one – in blue.

Methylene solution colors the cellulose cell walls in dark blue.

Test for starch grains

A few drops of Lugol solution was applied to the sections. The starch granules are stained purple-black.

Micropreparations were analyzed in *Mikos* optical microscope coupled with computer software.

3. Results and discussions

Morphology and biometry

Coffee senna (*Senna occidentalis* (L.) Link. or *S. occidentalis* (L.) Roxb.) belongs to the family Fabaceae. It is a pantropical plant species native to the tropical and subtropical regions of America (Colombia, Argentina, Ecuador, Brazil, Peru and Paraguay). The species was mentioned as invasive in parts of Kenya, Uganda and Tanzania (Global Invasive Species Database). Nowadays, Coffee senna is grown on extensive plantations for industrial purposes in India and Egypt (Sayed et al., 2016).

Coffee senna is a dicotyledonous, foetid, annual plant, which develops very strong and long root of tetrarch in nature. *S. occidentalis* is a pantropical plant species native to the Americas and under those conditions can be annual or undershrub (Rotton et al., 2021). Similarly, in many scientific works it is described as herb or undershrub (Kumar, 2009; Suma and Tanuja, 2014), but in the conditions of the Republic of Moldova, characterized with a temperate-steppe climate and differing from those of the native center of formation, the plant develops and grows as an annual. The stems are erect, green and brownish in color with a soft and smooth texture. Imparipinnate compound leaves with alternate arrangement on the stem are characterized by strong rachis and ovate to ovate-lanceolate leaflets. Fruits are pods, brown greenish in color, cylindrical, slightly curved with 30 or more brown to dark-olive green, hard, matt, and lenticular to cordate, slightly flattened seeds (**Fig. 1, 2**).

Coffee senna plant propagates through seeds and it is fast growing. Morphological description and biometric analysis of measurable and numerable parameters was applied to 50 plants. The diagnostic morphological characteristics of plant organs are specified in the **Table 1.**

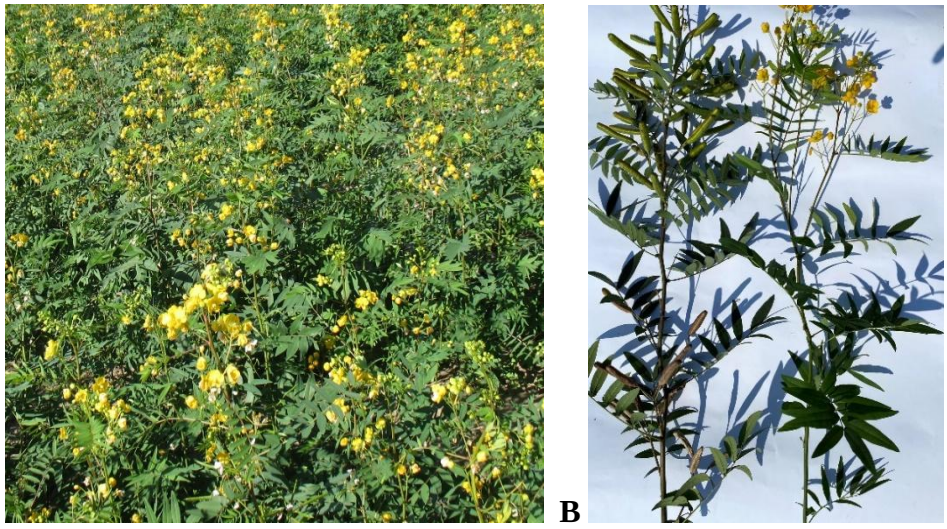

Fig.1. *Senna occidentalis* species: A – plants in the Collection of Medicinal and Aromatic of IGPhPP; B – morphology of plant

Fig. 2. Morphology of plant organs: A – roots; B – leaves; C – flowers; D – fruits and seeds

Table 1. Morphological characteristics of organs of *Senna occidentalis* species from IGPPP collection

Rows	Morphological parameters	Morphological characteristics
1.	Vegetative form of plant	Annual plant
2.	Root	Strong tap system
3.	Type of leaf	Imparipinnate compound with strong rachis
4.	Arrangement on the stem	Alternate
5.	Shape of rachis	Obovate, prominent wing type
6.	Shape o leaflet	Ovate to ovate-lanceolate
7.	Shape of leaflet apex	Slender acuminate
8.	Shape of leaflet base	Ovate, obtuse, sometimes asymmetric
9.	Venation of leaflets	Pinnate (mature leaflets with sunken veins)
10.	Shape of stipule	Triangular
11.	Flower	Yellow of papilionaceous type
12.	Type of inflorescence	Erect simple raceme
13.	Type of fruit	Pod with a rostrum at the apex, splits open along 2 thickened lines into 2 valves
14.	Shape of pod	Rounded (cylindrical) slightly curved
15.	Color of pod	Brown with a slightly greenish tinge
16.	Surface of pod	Matt with regular ribs perpendicular to the length of the pod
17.	Shape of seed	Lenticular to cordate, slightly flattened
18.	Color and surface of seed	Dark-olive green to brownish, mat, hard
19.	Smell of plant, especially of leaves	Foetid smell when damaged
20.	Usage Hazard	Toxic upon ingestion

We mentioned that the most of the morphological characteristics shown on plants in the Collection of Medicinal and Aromatic Plants of IGFPPl are consistent with morphological descriptions by other authors (Kumar, 2009; Naeem et al., 2009; Nassar et al., 2013; Fonkou et al., 2018).

The experimental data of *S. occidentalis* plantation over 5 years show that climatic conditions in the Republic of Moldova are favorable for their growth and development. Phenological observations denote that the plants develop healthy appearance and are not attacked by any pathogens. The plants being annuals go through the whole onto morphogenetic cycle of development during one vegetative period, which ends with fruit ripening and complete seed maturation. The biometric data results of the Coffee senna plants are shown in **Table 2**, which demonstrate the development peculiarities of the plants in the Collection of Medicinal and Aromatic Plants.

Anatomy of plant organs

The anatomical study was carried out on multiple micrographs, representing cross-sections or superficial views, and the highlighted structures were evaluated in the light of data from scientific papers already published by other authors and presented in **Table 3**.

Table 2. Biometric parameters of *Senna occidentalis* plants in the IGPhPP collection (2020-2021 years)

Rows	**Biometric parameters**	**Average value M±m**
1.	Height of plant (cm)	162.4±9.1
2.	Wide of the main stem: lower/ middle/ upper portions of plant	6.6±0.9/ 4.9±0.6/ 3.1±0.2
3.	Length of main root (cm)	56.6±4.1
4.	Number of leaves per axial twig	17.8±1.6
5.	Number of leaves plant	38.8±1.9
6.	Length of leaf (cm)	18.4±2.0
7.	Wide of leaf (cm)	12.5±1.3
8.	Length of leaflet (cm)	5.6±0.4
9.	Wide of leaflet (cm)	2.3±0.2
10.	Number of pair leaflets per leaf	5.2±0.8
11.	Number of inflorescences per plant	25.2±1.2
12.	Number of flowers per inflorescence	19.3±1.8
13.	Length of inflorescence (cm)	11.8±1.1
14.	Number of pods per plant	63.3±7.7
15.	Length of pod (cm)	6.5±0.5
16.	Wide of pod (cm)	0.9±0.1
17.	Number of seed per pod	32.5±2.1
18.	Length of seed (mm)	4.5±0.3
19.	Wide of seed (mm)	3.5±0.2
20.	1000 seed weight (g)	17.5±0.9

Table 3. Anatomical characteristics for *Sennia occidentalis* species in current research and literature data

Organs	**Anatomical parameters**	**Our results**	**Other researches**	
Root	Tetrarch type	+	+	Nassar et. al., 2011
	Diarch type		+	Kumar, 2009
	Druses in cortex and Pismatic crystals in the vascular sheath	+		
Stem	Single layered epidermis with thin cuticle		+	Suma and Tanuja, 2014
	Uni- and multicellular non-glandular trichomes	+		
	Glandular trichomes	+		
	Paracytic stomata	+		
	Druses and solitary prismatic crystals in cortex	+	+	Suma and Tanuja, 2014; Nassar et al., 2013
	Prismatic crystals in the vascular sheath	+		
	Druses in the pith		+	Nassar et al., 2013

Leaf	**Rachis**	Uniseriate epidermis of rectangular cells	+	+	Nassar et al., 2013
		Unicellular non-glandular trichomes	+	+	Nassar et al., 2013; Ogundipe et al., 2009
		Multicellular non-glandular trichomes	+	+	Nassar et al., 2013
		Glandular trichomes	+	+	Nassar et al., 2013; Doty et al., 2020; Amponsah et al., 2016
		Paracytic, anisocytic and anomocytic stomata	+		
		Druses		+	Nassar et al., 2013
		Prismatic crystals in the vascular sheath and druses	+		
	Blade leaflet	Dorsoventral mesophyll	+	+	Nassar et al., 2013; Suma and Tanuja, 2014; Amponsah et al., 2016
		Solitary crystals and druses	+	+	Nassar et al., 2013
		Prismatic crystals in the vascular sheath	+		
		Upper epidermis of thin-walled cells		+	Nassar et al., 2013; Suma and Tanuja, 2014
		Lower epidermis of radially elongated cells		+	Nassar et al., 2013
		Wavy epidermal cells	+	+	Amponsah et al., 2016; Kotresha and Seetharam, 2000
		Lower epidermis of rectangular cells		+	Suma and Tanuja, 2014
		Amphystomatic leaf	+	+	Nassar et al., 2013; Amponsah et al., 2016; Suma and Tanuja, 2014
		Paracytic stomata on both epidermises	+	+	Suma and Tanuja, 2014; Kotresha and Seetharam, 2000
		Anomocytic stomata	+	+	Amponsah et al., 2016
		Paracytic and anisocytic stomata	+	+	Ogundipe et al., 2009; Nassar et al., 2013
		Paracytic, anisocytic and tetracytic stomata	+	+	Begum et al., 2014
		Paracytic and anomocytic stomata		+	Kumar, 2009
		Horn like unicellular non-glandular trichomes	+	+	Suma and Tanuja, 2014; Amponsah et al., 2016; Kotresha and Seetharam, 2000
		Multicellular non-glandular trichomes	+		
		Only glandular trichomes		+	Begum et al., 2014; Doty et al., 2020; Kotresha, 2000
Flower	**Sepal**	Upper epidermis of a uniseriate layer of barrel-shaped cells		+	Nassar et al., 2013
		Upper epidermis with a thin cuticle layer		+	Nassar et al., 2013
		Paracytic and anisocytic stomata	+		
		Filiform formations	+		
		Glandular trichomes	+		
	Petal	2 epidermal layers with nearly square parenchyma cells		+	Nassar et al., 2013

		Mamelous protuberance	+		
		Paracytic stomata	+		
		Folded surface	+		
		Glandular trichomes	+		
Pericarp fruit		Exocarp consisting of epidermis only with uniseriate, barrel-shaped cells	+	+	Nassar et al., 2013
		Waxes deposition and thick layer of epidermis cuticle	+	+	Nassar et al., 2013
		Mesocarp consisting of 10 layers of irregular, thin-walled parenchyma cells, sometimes slightly lignified	+	+	Nassar et al., 2013
		Endocarp of uniseriate, cylindrical parenchyma cells	+	+	Nassar et al., 2013
		Druses	+		
		Non-glandular and glandular trichomes	+		
Seed		Coat formed from 4 distinct layers	+	+	Nassar et al., 2013
		Outermost layer of seed coat composed of waxy cuticle	+	+	Nassar et al., 2013
		Second layer is formed of thick-walled, elongated palisade cells called macrosclereids	+	+	Nassar et al., 2013
		Two cotyledons are more or less orbicular and occur parallel position to the long axis of the embryo	+	+	Nassar et al., 2013
		Lipidic globules in cotyledons	+		

Root anatomy (**Fig. 3**). Anatomical structure was investigated on cross-section of the mature main root. Secondary structure is established by secondary growth. On cross-section, the first histological zone is the rhizodermis, consisting of one layer of cells. We can also see the formation of peridermis, composed of several layers of phellem, phellogen (cork cambium) and phelloderm. The periderm provides safe protection for the internal root structures. In section, the periderm is followed in the interior by cortex with druses in some cells, and islands of primary phloem and clusters of sieve tubes with their accompanying cells, in addition to sclerenchyma fibers and phloem parenchyma, which form the secondary phloem, located opposite to the rows of secondary xylem. In the vascular bundles sheath, there are prismatic crystals. The vascular cambium is thin and consists of 3-4 layers of meristematic cells. A large secondary xylem space is mentioned, represented by vessels in radial rows, surrounded by sclerenchyma fibers and separated by parenchyma cells. The primary xylem occupies the central position of the root. The prismatic crystals of calcium oxalate are present in the vascular bundle sheath. Data on the anatomy of the main root in *C. occidentalis* are largely consistent with those described in other works (Kumar, 2009; Nassar et al., 2011). Confusion is found in the type of vascular bundle, where we describe it as tetrarch (4 patches of xylem alternate with similar number of phloem patches), which is in agreement with Nassar's (2011) description, but disagrees with Kumar (2009), who mentions it as diarchic. The roots give a reddish stain when tested for anthraquinones, being one of the best effects comparatively with other plant organs (**Fig. 4**).

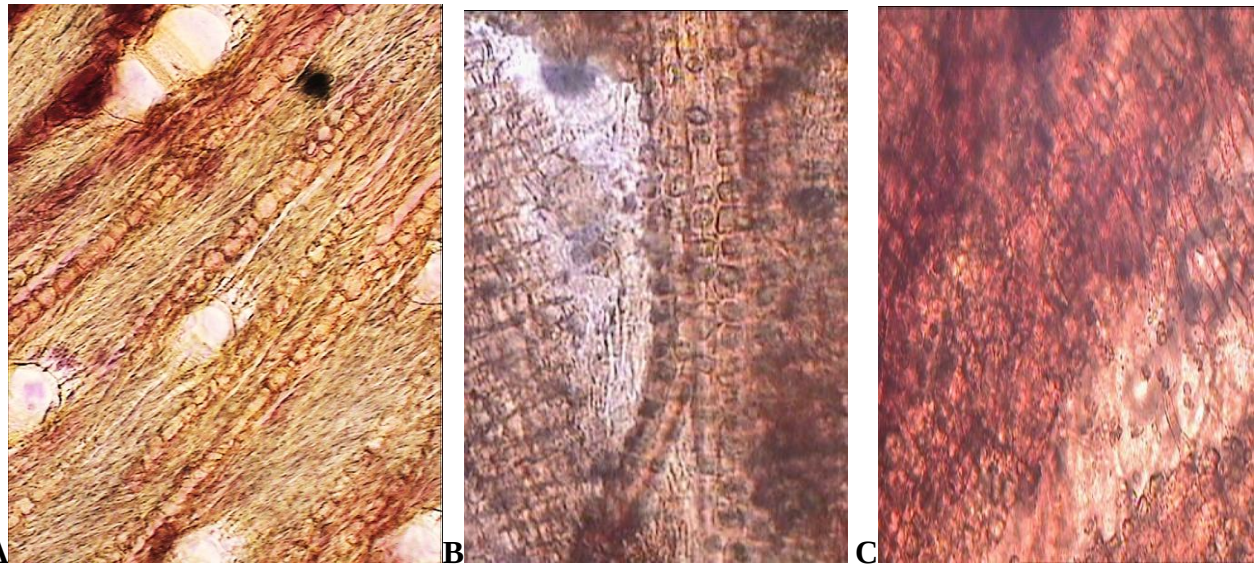

Fig. 3. Anatomy structure of root (40x): A – lignified rows of xylem (staining with Cl-Zn-I solution); B – prismatic crystals in vascular bundles sheath; C – reddish effect of anthraquinone test

Fig. 4. The effect of the anthraquinone test on plant organs: A – roots; B, C – stems; D – leaf rachis; E – leaflets; F – flowers; G – pods; H – fruit pericarp; I – seeds

Stem anatomy (**Fig. 5**). Anatomical structure was investigated on cross-section of the middle internode of the mature main stem. The cross-section configuration of the stem is circular, with attenuated and not prominent ribs. The anatomical structure is the result of secondary thickening, mainly expressed by the massive secondary xylem in the central cylinder. The outer layer of the stem is epidermis, composed of radially and tangentially elongated cells covered by a thick layer of cuticle. Paracytic stomata are present. Non-glandular unicellular and multicellular trichomes are found very rarely. Glandular trichomes, consisting from short stalk and multicellular glands with brownish content have a moderate frequency. The cortex composed of 6-12 layers is represented by 4-8 layers of collenchymatous cells and 2-3 layers of parenchymatous chlorenchymatous cells.

In epidermis and cortical cells, druses and solitary prismatic crystals were observed, but we note, that prismatic crystals are more characteristic for vascular bundles sheath.

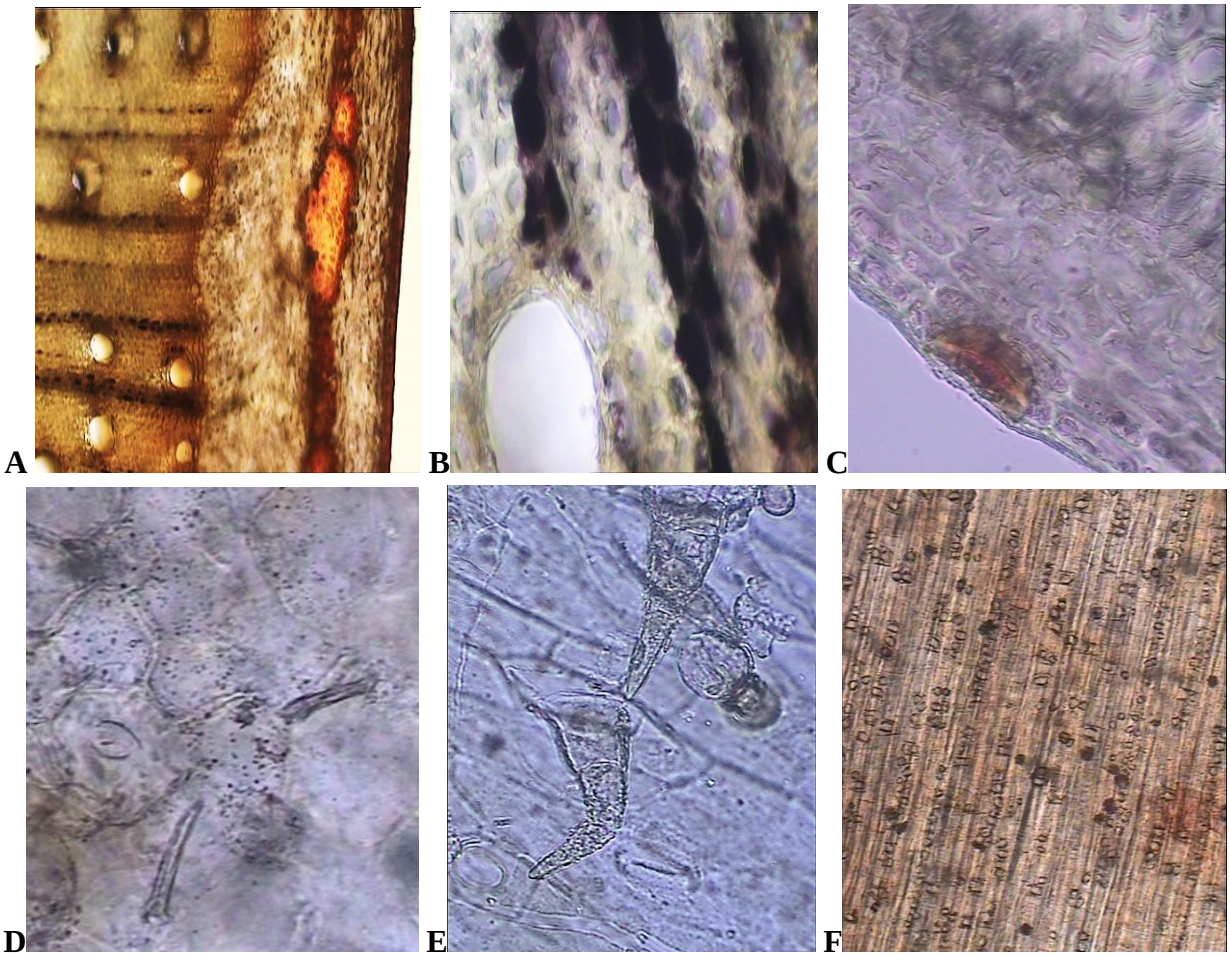

Fig. 5. Anatomy structure of stem (40x): A – histological zonality on cross-section: 1 – epidermis, 2 – cortex, 3 – sclerenchyma fibers, 4 – phloem, 5 – secondary xylem; B – parenchimal rows with starch grains (staining with Lugol solution); C – outerlayers of stem with brownish gland (cross-section); D – unicellular non-gladular trichomes (upper view); E – multicellular non-glandular trichomes (upper view); F – prismatic crystals

Other authors (Suma and Tanuja, 2014) indicated the presence of both prismatic crystals and druses (rosette) in cortical cells, and prismatic solitary crystals only in the stem endodermis.

The central cylinder is surrounded by an interrupted ring of sclerenchymatous fibers. In the central cylinder there are about 25 collateral open vascular bundles of various sizes, arranged in a ring shape. The secondary phloem consists of sieve tubes, companion cells, phloem parenchyma, while sclerenchymatous phloem fibers are not observed. The secondary xylem contains a lot of vessels of different diameters, sclerenchyma fibers and parenchyma cells. The primary xylem forms a thin, pressed layer attached to the pith. Medullary rays from lignified parenchyma cells separate the vascular bundles. The pith, consisting of polygonal parenchymatous cells, occurs the central part of the stem. The druses were mentioned like other authors (Nassar et al., 2013).

Leaf. *Rachis anatomy* (**Fig. 6**)**.** In cross-section, the petiole is cordate in shape, with a groove protruding inward and two prominent notches on the adaxial side. The petiole is surrounded on the outside by a uniseriate epidermis, covered by the cuticle. In the

epidermis, the paracytic (each guard cell is accompanied by 2 cells, whose longitudinal axes are parallel to those of the guard cells and the aperture), anysocytic (guard cells are surrounded by 3 subsidiary cells of unequal size) and anomocytic (guard cells are surrounded by 5-6 cells with radial position) stomata are observed.

Rarely, non-glandular, unicellular trichomes are found. Also, the glandular trichomes with multicellular, brownish glands were observed, mentioned early in other works (Nassar et al., 2013; Amponsah et al., 2016; Doty et al., 2020).

In general, data on the presence of different types of trichomes in *S. occidentalis* rachis leaf are contradictory. Some anatomical studies (Begum et al., 2014; Doty et al., 2020) describe only multicellular glandular trichomes, while other studies (Ogundipe et al., 2009; Saheed and Illoh, 2010) mentioned only the presence of unicellular non-glandular trichomes. Another anatomical research (Nassar et al., 2013) describes the presence of both unicellular and multicellular non-glandular trichomes, although the latter with a rare frequency. Below the epidermis, there is cortex composed of chlorenchyma (most on the adaxial side) and collenchyma.

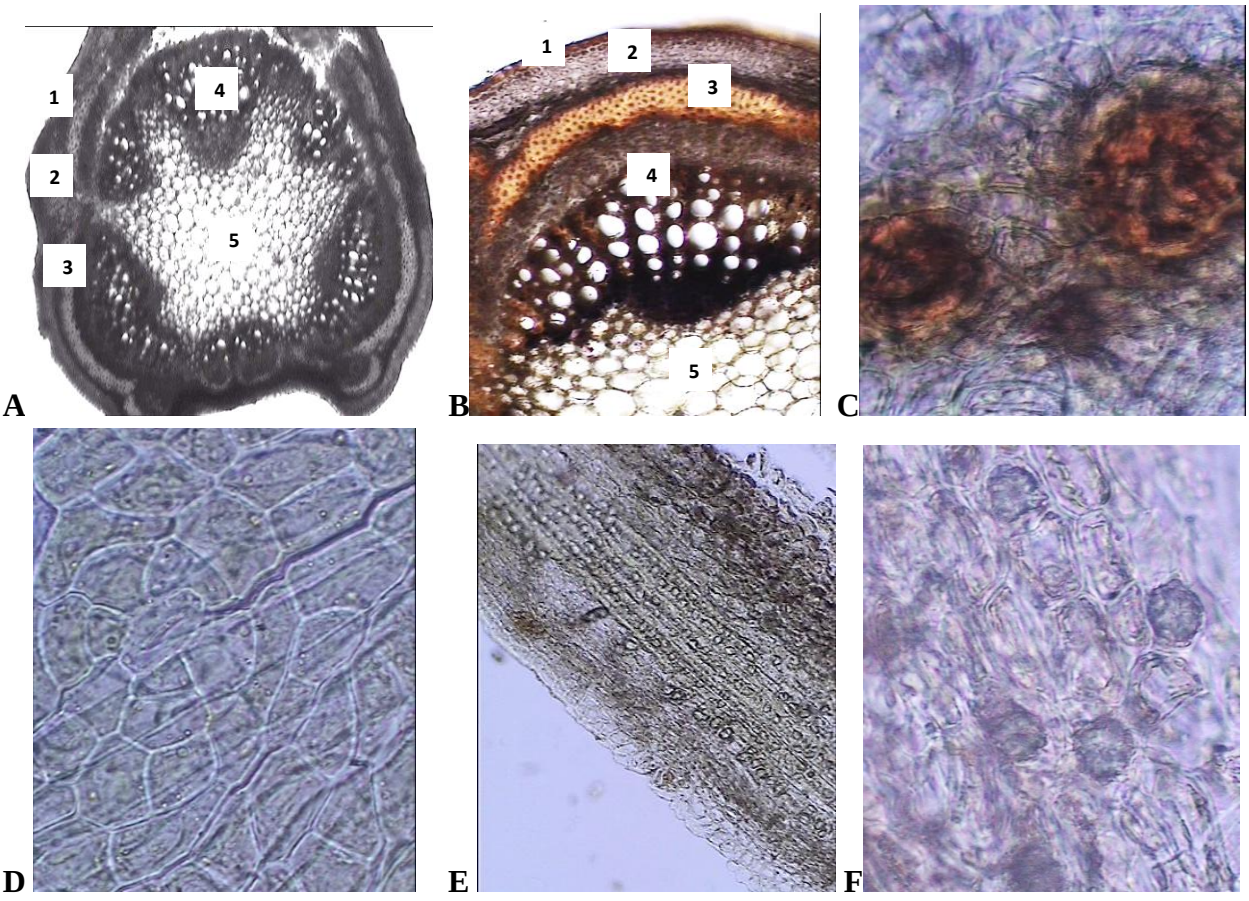

Fig. 6. Anatomy aspects of leaf rachis: A (10x), B (staining with Zn-Cl-I solution, 40x) – cross-section of rachis: 1 – epidermis, 2 – chlorenchyma, 3 – collenchyma, 4 – collateral vascular bundle, 5 – mesophyll; C – glandular trichomes with brownish content (upper view, 40x); D – anomocytic stomata (upper view, 40x); E – sheath of vascular bundles with prismatic crystals (10x); F – druses in parenchyma cells (40x)

Marginally, on the central part of the petiole there are 7 collateral conducting bundles (3 dossals, 2 laterals and 2 ventrals) with well-developed sclerenchyma sheath, especially on external side, which induces stringency for rachis, the pith is wide and is represented by assimilating parenchyma. There are 2 ridge bundles. The druses and prismatic crystals are mentioned. The presence of druses only, has been mentioned in another research (Nassar et al., 2013).

The leaflet blade anatomy (**Fig. 7**). According to the imparipinnate compound type of the leaf, the anatomical study was carried out on leaflets from the central region of the mature leaf. The anatomical type of the leaf is the dorsoventral mesophyll, in which the palisade tissue is located on the adaxial side and the spongy tissue on the abaxial side of the blade. The adaxial epidermis is composed of thin walled, tangentially elongated cells covered by a layer of cuticles. The abaxial epidermis consists of thin walled, radially elongated cells and is thinner than the adaxial epidermis. This data concerning the anatomy type of leaf is in harmony with results found in some articles (Kumar, 2009; Nassar et al., 2013).

It is an amphistomatous leaf, as stomata are found on both epidermises, but there are more numerous on the abaxial than on the adaxial side. Note, that the paracytic type of stomata was found on both types of epidermis, the anisocytic type and tetracytic (guard cells are surrounded by 4 subsidiary cells) ones, only on the abaxial one. These data are consistent with those reported in other articles (Begum et al., 2014) and differ from those described in another papers (Ogundipe et al., 2009; Nassar et al., 2013) which indicate the presence of 2 types of stomata (paracytic and anisocytic) on the abaxial epidermis and only the paracytic one on the adaxial side, while in another paper (Kotresha and Seetharam, 2000; Suma and Tanuja, 2014) it is described only the paracytic type, but on the both leaf epidermises, however in this research (Amponsah et al., 2016) – only anomocytic stomata on both sides of the leaf.

Both epidermises develop short, non-glandular, horn like unicellular trichomes, but more on the abaxial epidermis, particularly along the nerves and in the basal part of the leaflet, and multicellular non-glandular ones only on the abaxial side. The morphology of horn non-glandular trichomes is similar to that described in this scientific paper (Suma and Tanuja, 2014). The multicellular glandular trichomes with brownish content observed on both epidermises of leaflet, but frequently on the abaxial one was pointed by other scientific works (Kotresha and Seetharam, 2000; Begum et al., 2014).

Chlorenchymatous palisade tissue consists of 1-2 layers and occurs a half of mesophyll. Another half of mesophyll occurs the spongy tissue consisted of lobed chlorenchymatous cells with large intercellular spaces. The blade is perforated by vascular bundles, one principal, large, located in the midrib and others of much smaller diameter, distributed in the sides of the blade. The mechanical tissue, represented by the collenchyma is mentioned on the side of principal vascular bundle and is prominent on the abaxial side of the blade.

The sclerenchymatous cells surround as a continuous ring the midrib, which is thicker on the abaxial side. Thus, the main vascular bundle is not directly embedded by the mesophyll as are the other side small vascular bundles. The vascular bundles are collateral, the xylem represented by vessels is located on the adaxial side and phloem – by sieve tubes, companion cells and parenchyma, are found on the abaxial side. Solitary crystals and druses of calcium oxalate are distributed throughout in the mesophyll, but only prismatic ones are in rows in the sheath of the vascular bundles.

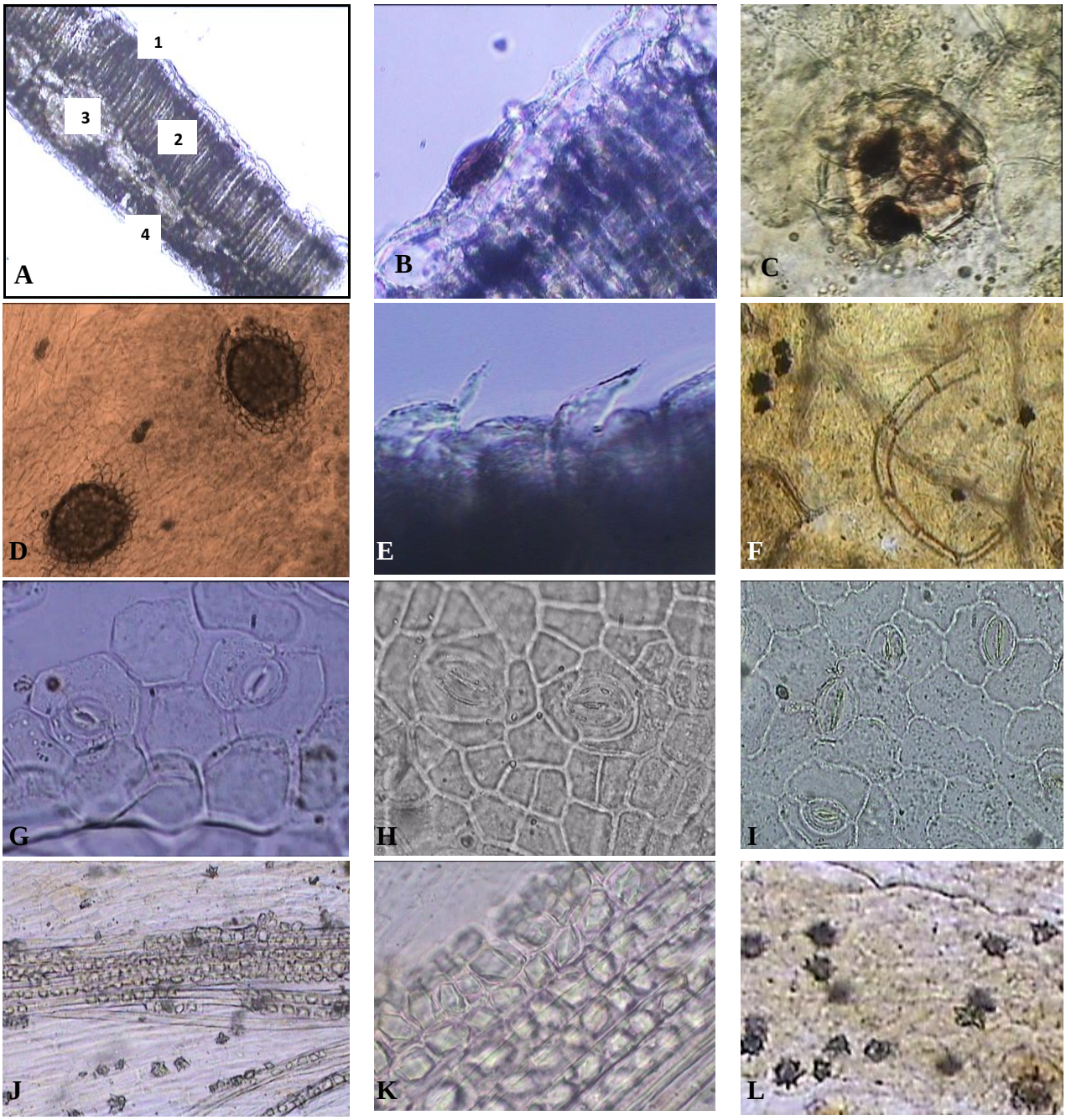

Fig. 7. Anatomy aspects of leaflet: A – dorsoventral type of mesophyll on cross-section (10x): 1 – adaxial epidermis, 2 – palisade mesophyll, 3 – spongy mesophyll, 4 – abaxial epidermis; B – uniseriate epidermis with glandular trichome in cross-section (40x); C – glandular trichome with brownish content (upper view, 40x); D – glandular trichome with brownish content (upper view, 10x); E – short unicellular non-glandular trichomes (40x); F – multicellular non-glandular trichomes (10x); G – paracytic stomata (upper view, 40x); H – anisocytic stomata (upper view, 40x); I – paracytic, anisocytic and anomocytic stomata (upper view, 40x); J – druses and prismatic crystals (upper view, 10x); K – prismatic crystals in the sheath of vascular bundles (40x); L – druses in mesophyll (40x).

Leaf anatomy regarding the species *S. occidentalis* is generally consistent with a study of Nassar et al. (2013), except for calcium oxalate crystals, mentioned as solitary and druses.

Leaves give the lowest expression of the staining gradient for anthraquinone testing compared to fruits, seeds, and roots.

Flower anatomy (**Fig. 8, 9**). Investigation of flower anatomy was carried out on sepals and petals. Two marginal layers of uniseriate epidermis, covered by a thin layer of cuticles, can be distinguished on cross-section of the sepals. The epidermises contain paracytic and rarely anisocytic stomata of rounded shape protruding from the level of the epidermis, from which radially arranged folds can be seen. Several layers of chlorenchymatous cells with large intercellular spaces, perforated by small vascular bundles, fill the space between the epidermis.

Two very thin layers of epidermis, made up of square, tightly packed cells, can also be seen in cross-section of the petals. On the upper epidermis there are mamelous protuberances, especially along the veins. The folding of the epidermis surfaces is radial around the rounded paracytic stomata. On the base of the petals, there are developed a few small and thin non-glandular trichomes.

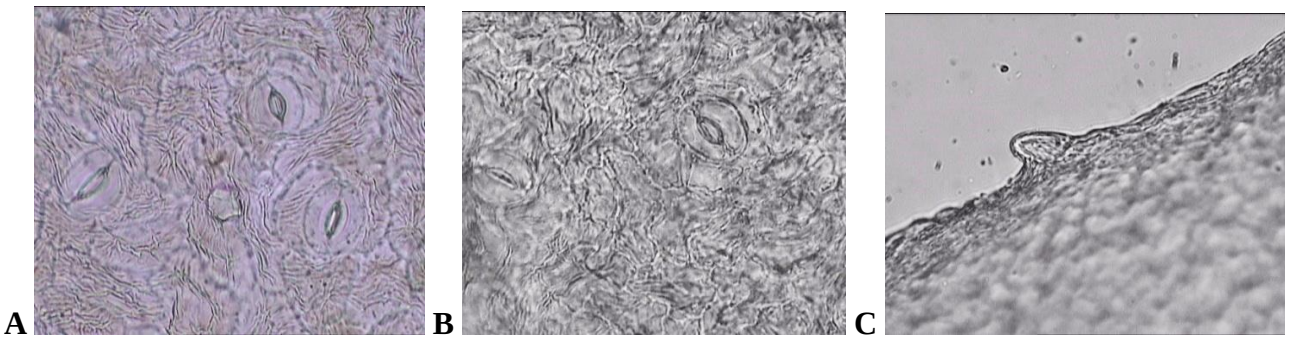

Fig. 8. Sepals anatomy: A – rounded paracytic stomata on adaxial epidermis (40x); B – rounded paracityc stomata on abaxial epidermis (40x); C – short non-glandular trichome on abaxial epidermis (cross-section, 40x)

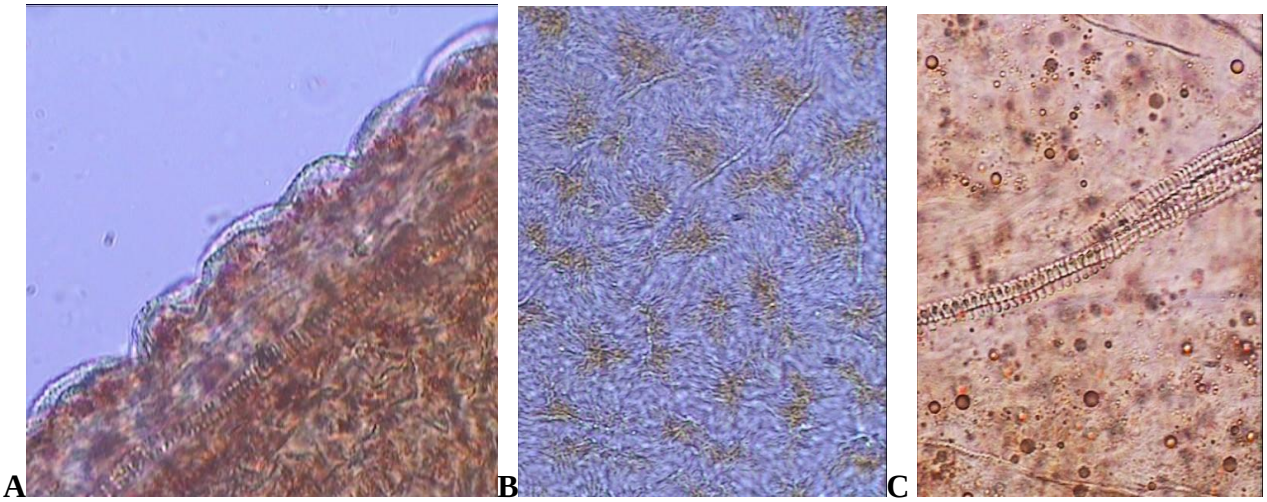

Fig. 9. Petals anatomy: A – mamelous protuberances on cross section (40x); B – mamelous protuberances (upper view, 40x); C – lipidic globules (staining with Sudan III, 40x)

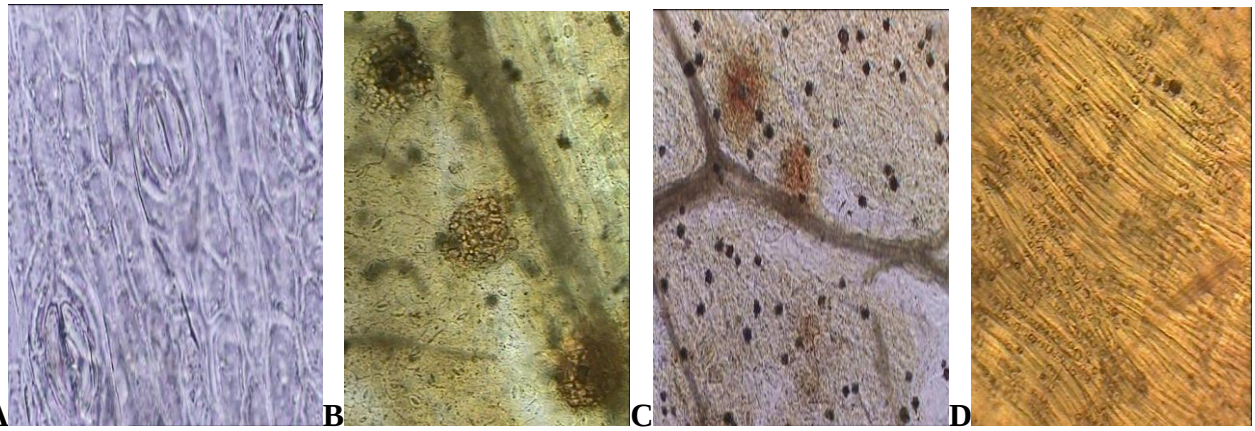

Fig. 10. Outer epidermis of pericarp (upper view): A – paracytic stomata (40x); B – glandular trichomes with multicellular, brownish glands (10x); C – druses (10x); D – prismatic crystals (10x)

Fig. 11. Anatomy of seed: A – seed coat (cross-section, 10x); B – compact arrangement of epidermis cells (upper view, 10x); C – lipidic globules in cotyledons (staining with Sudan, 40x)

Between the epidermis, there is the parenchyma consisting of 3-5 layers of cells, frequently developing lipid-containing globules (red staining when Sudan III reagent is applied). Sepals and petals give a reddish stain when tested for anthraquinones.

Fruit pericarp anatomy (**Fig. 10**). Three histological zones can be distinguished on cross-section of the pericarp in the middle area of the pod: exocarp, consisting only of a layer of tangentially elongated outer epidermal cells, with thickened and slightly lignified cell walls, compact, rarely separated by paracytic stomata and very rarely by unicellular non-glandular trichomes, covered by a relatively thick cuticle; mesocarp is composed of 8-10 layers of parenchymal cells, irregular in shape, in some of which there are druses; the mesocarp is perforated by collateral vascular bundles, surrounded by the fibrous sclerenchymatous sheath; endocarp, represented by the inner uniseriate epidermis, consisting by cells of the same shape, is compactly arranged.

Seed anatomy (**Fig. 11**). The seed consists of seed coat and embryo. The cross-section of the seed coat shows: the outer layer is composed of thick waxy cuticle, next there is epidermis, consisting of thick-walled, tangentially elongated cells with a very compact arrangement, followed by several layers of osteosclereids, and the last inner layer is represented by the parenchyma, consisting of thin walled, tangentially elongated cells. The embryo includes 2 cotyledons, extending parallel to the long axis, formed by the radical, hypocotyl, epicotyl and plumule. The

cotyledons are covered by epidermis and the internal mesophyll consists of parenchymatous cells with gelatinous content and small intercellular spaces. The anatomy description of seed structure of *S. occidentalis* agrees with the work of Nassar et al. (2013) and another study on species of *Cassia* genus (Sahai, 2001).

Our results show that, even though full sun and moderate water are required for the care and propagation of *S. occidentalis*, however, under the conditions of the steppe climate of the Republic of Moldova, with moisture deficit practically all year round, the plants in the Collection of Medicinal and Aromatic Plants have developed very well, both underground and above ground parts.

Notwithstanding the different and sometimes contradictory morphological and anatomical data in the literature, we were nevertheless able to evaluate the data obtained in the current study through the prism of those already presented by other scientific researchers (Ogundipe et al., 2009; Saheed and Illoh, 2010; Nassar et al., 2013; Doty et al., 2020) and to establish specific structural indices for each organ of Coffe senna plants. Anatomical study and consultation of literature shows that most conflicting data refer to the type of stomata on the aerial parts of Coffe seena plants: paracytic type only (Suma and Tanuja, 2014); paracytic and anisocytic type (Ogundipe et al., 2009; Nassar et al., 2013); paracytic, anisocytic and tetracytic type (Begum et al., 2014); anomocytic only (Amponsah et al., 2016); paracytic and anomocytic type (Kumar, 2009). We found on aerial parts of plants paracytic, anisocytic and anomocytic type, but with different frequency and distribution, however, most encountered being the paracytic type.

It is known that anatomical structures were established during evolution and represent specific structures of species. Anatomical structures are stable and conservative, depending on the growing conditions of the plant, only the quantitative aspects of the structures can change. The slightly different information on the anatomical structures of sp. *S. occidentalis* in the literature requires some explanation. We could assume that microscopic studies were carried out at different stages of ontogenetic development of the plant, but it was not specified in the paper, or the elucidation of all structures certainly depends on the complexity and insistence of analysis of the applied microscopic techniques. As an example, non-glandular trichomes occur very rarely, only on the rachis and the basal part of the leaf blade and could be identified by analysis of micropreparations of cross-sections on fresh material and of leaf surfaces obtained from clarified botanical material in hydrochloride solution.

The taxonomic value of anatomical characters, especially the epidermal ones of leaves, has a decisive weight in taxonomic monographs (Rejdali, 1991). In this context, microscopic studies to evaluate and highlight those with diagnostic role in the identification with certainty the species and in determination of structural indices with adaptive role under conditions of global climate change are very necessary. For example, the physiological role of calcium oxalate crystals in plants is still debatable. A role of these crystals against herbivores is known, but lately they are increasingly thought to play a key role in several biological processes in plants, such as: oxalate functions in metal tolerance, ion balance and defense against pathogenic bacteria, viruses, fungi, and insects (Franceschi and Nakata (2005); Foster et al., 2016). However, recently, more and more authors attribute the role of calcium oxalate crystals in plant adaptation and protection under adverse climatic factors especially related to the evolutionary adaptation of species to drought

stress conditions (Leon-Martinez and Ortiz-Hernandez, 2022).

In summary, this morpho-anatomical research will reveal new aspects, will contribute to support some anatomical features already reported but less confirmed in the studies of the last decades on the species *S. occidentalis* and will serve as landmarks for a real clarity of the integral microstructural picture.

Conclusions

1. The present study shows that microscopic research on the species *S. occidentalis* is very welcome and important, as in scientific research there are often conflicting and confusing data. Anatomical research is needed to highlight diagnostic microscopic characters specific to each plant organ, to identify with certainty the taxonomy of species *S. occidentalis* and the relationships between members of the genus *Senna*, considering that many species are potentially toxic.
2. Specific microscopic parameters for *S. occidentalis* species are: tetrarch root type; secondary anatomical structure of root and stem; unicellular non-glandular trichomes on stem, rachis and leaflet base; glandular trichomes with brownish content on stem, leaves and floral elements; paracytic, anysocytic and anomocytic stomata on aerial part of plants; calcium oxalate in the form of prismatic crystals, usually in the sheath of vascular bundles and druses in the parenchymatic cells of roots, stems and leaves; presence of lipid globules in petals and cotyledons of seeds; presence of anthraquinones with different gradation (decreasing) – roots, pods, seeds, flowers, leaves, stems.

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.

Acknowledgement

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References

1. Allsopp R (1996) Dictionary of Caribbean English Usage. pp 441-604, ISBN 9789766401450.
2. Amponsah IK, Mensah AY, Ampofo EK, Bekoe SO, Sarpong FM, & Jibira Y (2016) Pharmacognostic studies of the leaves and seeds of *Cassia occidentalis* (Linn.) (Leguminosae). J of Pharmacognosy and Phytochemistry, 5(3), p 250
3. Begum A, Rahman MO, & Begum M (2014) Stomatal and trichome diversity in Senna Mill. from Bangladesh. Bangladesh J of Plant Taxonomy, 21(1), pp 43-51
4. Calalb T, Nistreanu A (2021) Botanica farmaceutică. Compendiu pentru lucrări de laborator, Print-Caro SRL, pp 19-29
5. Doty MK, Rashid P, & Shethi KJ (2020) Study of petiole anatomy and pollen morphology of five species of Senna Mill. from Bangladesh. Dhaka University J of Biological Sciences, 29(2), pp 245-252

6. Fonkou MFY, Kamdem JP, Fono LA, & Priso RJ (2018) Identification keys of seven cassia species from the (Caesalpinioideae: Fabaceae)
7. Foster J, Luo B, Nakata P (2016) An oxalyl-coa dependent pathway of oxalate catabolism plays a role in regulating calcium oxalate crystal accumulation and defending against oxalate-secreting phytopathogens in *Medicago truncatula*. PLoS ONE 11(2), pp 1-15 DOI:10.1371/journal.pone.0149850
8. Franceschi VR, Nakata PA (2005) Calcium Oxalate in Plants: Formation and Function. Annual Review of Plant Biology, 5, pp 641-671
9. Khare CP (2004). Encyclopedia of Indian Medicinal plants. Springer publication, 620 p
10. Kotresha K, Seetharam YN (2000) Epidermal micromorphology of some species of *Cassia* L. (Caesalpinaceae), Phytomorphology 50 (3, 4), pp 229-237
11. Kumar A (2009). *Cassia occidentalis* Linn. Morphological and anatomical study. Science 2.0, ION Pblications L.C. Published on Science 2.0 (https://www.science20.com) Source URL: https://www.science20.com/humboldt_fellow_and_science/blog/cassia_occidentalis_linn_morphological_and_anatomical_study
12. Leon-Martínez FM, Ortiz-Hernandez YD (2022) Microscopical observations of crystal deposits on the epidermis of leaves of *Agave potatorum.* Micron 154, pp 1-9 https://doi.org/10.1016/j.micron.2021.103201
13. Naeem M, Masroor M, Khan A (2009) Phosphorus ameliorates crop productivity, photosynthesis, nitrate reductase activity and nutrient accumulation in coffee senna (*Senna occidentalis* L.) under phosphorus-deficient soil, J of Plant Interactions, 4:2, 145-153 Doi: 10.1080/17429140802193178
14. Nassar MAA, Ramadan HRH, & Ibrahim HMS (2013) Anatomical structures of vegetative and reproductive growth of *Senna occidentalis* (L.) Link (Caesalpiniaceae). Turkish J of Botany, 37(3), pp 542-552
15. Nassar MAA, Ramadan HRH, Ibrahim HMS (2011) Morphological characteristics of vegetative and reproductive organs of *Senna occidentalis* (Caesalpiniaceae). Research J of Agriculture and Biological Sciences, 7, pp 260-270
16. Ngombe N, Ngolo C, Kialengila D, Wamba A, Mungisthi P, Tshibangu P, Dibungi P, Kantola P and Kapepula P (2019) Cellular Antioxidant Activity and Peroxidase Inhibition of Infusions from Different Aerial Parts of *Cassia occidentalis*. J of Biosciences and Medicines, 7, 83-94. doi: 10.4236/jbm.2019.74009
17. Nistreanu A, Calalb T (2016) Analiza farmacognostică. Chișinău, Tipog.”Elan Poligraf”, 316 p
18. Ogundipe OT, Kadiri AB, Adekanmbi OH (2009) Foliar epidermal morphology of some Nigerian species of *Senna* (Caesalpiniaceae). Indian J of Science and Technology 2, pp 5-10
19. Rejdali M (1991) Leaf micromorphology and taxonomy of North African species of *Sideritis* L. (Lamiaceae). Bot. J. linn. Soc. 107, pp 67-77
20. Rotton H, Klitgård B (2021) *Senna occidentalis* (L.) Link. In: The IUCN Red List of Threatened Species 2021 https://doi.org/10.2305/IUCN.UK.2021-2.RLTS.T130525346A158506718.en. Accessed 21 June 2022
21. Sahai K (2001) Anatomical variability in seed coat of some *Cassia* L. (Caesalpinioideae) species with taxonomic significance. Taiwania 46, pp 158-166
22. Saheed SA, Illoh HC (2010) A taxonomic study of some species in Cassiinae

(Leguminosae) using leaf epidermal characters. Notulae Botanicae Horti Agrobotici Cluj-Napoca, 38(1), pp 21-27

23. Sayed H, Ramadan M, Sayed M et al. (2016) Chemical constituents and hepatoprotective effect of *Cassia occidentalis* L. cultivated in Egypt. Assiut Vet Med J, Vol 62, No 149, pp 139-148
24. Singh VK, Jain J, Mishra AK (2016) Pharmacological and phytochemical profile of *Cassia occidentalis* L: A review. J of Drug Delivery & Therapeutics, 6(5), pp 91-96.
25. Suma M, Tanuja N (2014) Kasamarda (*Cassia occidentalis* Linn.), macro/microscopic profile. 2014. J of Biological & Scientific Opinion. 2 (1), pp 83-85. Doi.org/10.7897/2321-6328.02118
26. Vijayalakshmi S, Ranjitha J, Devi Rajeswari V, Bhagiyalakshmi M (2013) Pharmacological profile of *Cassia occidentalis* L – A review. Int J Pharm Sci, Vol 5, Issue 3, pp 29-33
27. Yadav JP, Arya V, Yadav S, Panghal M, Kumar S, Dhankhar S (2010) *Cassia occidentalis* L.: A review on its ethnobotany, phytochemical and pharmacological profile. Fitoterapia, 81(4), pp 223-230.
doi: 10.1016/j.fitote.2009.09.008.

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STIMULATION OF PHYSIOLOGICAL PROCESSES IN ST. JOHN'S WORT (*HYPERICUM PERFORATUM* L.) SEEDLINGS BY TREATMENTS WITH TRIACONTANOL AND BENZYLADENINE

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Abstract: Treatment of St. John's wort plantlets with 1 µM triacontanol and 2 µM benzyladenine stimulates growth and metabolic processes, being an environmental-friendly approach for optimizing the cultivation of these valuable medicinal plants under controlled conditions. When the two growth regulators (a bioactive cuticular wax constituent and a cytokinin) are applied simultaneously, they act synergistically, enhancing each other's effect on the biomass accumulation and on certain parameters of the photosynthetic light use efficiency, such as the effective quantum yield of photosystem II and the overall vitality index of the photosynthetic apparatus which performs the conversion of light energy into usable forms for carbon dioxide assimilation. The results concerning the interactions between the two externally applied growth regulators during the early development of St. John's wort plants may lead to a more efficient cultivation of this herbal medicinal product, including the possibility to modulate the production of pharmacologically active metabolites.

Keywords: biomass production, carotenoids, chlorophyll fluorescence, growth regulators, photosynthetic quantum yield

1. Introduction

Cultivation of medicinal plants ensures better predictability and control of the production of pharmacologically active compounds, because plant growth and metabolism can be modulated by the culture conditions and by selected treatments (Tsukagoshi and Yamori, 2020). At present, herbal medicines suitable for complementary and alternative medicine are used at an increasing scale around the world, as ethnopharmacological traditions become more and more supported by scientific evidence regarding the biochemical composition of herbs and the action mechanisms of the different plant metabolites. In most of the European countries, plants used for registered herbal medicinal products are grown under controlled agricultural conditions (cultivated), excluding any contamination with pesticides and use of genetically modified plants (Edwards et al., 2015). Under these circumstances, the production of medicinal plants can be optimized in an environmental-friendly and cost-effective man-

ner by priming or hardening the plant with exogenous application of very small concentrations of bioactive substances. These compounds are able to induce metabolic changes that stimulate growth and tolerance to adverse environmental conditions, especially during the most sensitive periods of the plant's life cycle, such as germination and early vegetative development. The use of such growth regulators may significantly increase biomass production of medicinal plant, also leading to a more intense biosynthesis of the pharmacologically active metabolites (Garcia-Garcia et al., 2020; Godoy et al., 2021). From among the many bioactive compounds that may stimulate plant growth and metabolism both under normal and extreme (stressful) conditions, triacontanol is a natural constituent of the epicuticular waxes present on the surface of aboveground plant organs with primary structure, while benzyladenine is a synthetic cytokinin, being the cheapest among the representatives of this group of plant hormones (Naeem et al., 2012; Oshchepkov et al., 2020).

The growth regulating properties of triacontanol (Tria) were recently discovered as compared to most of the plant bioregulators, and its action mechanisms are still largely unknown. Recent studies have demonstrated that when this natural wax constituent of the plant cuticle comes in contact with a certain receptor in the plasma membrane, it induces the formation of secondary messengers (such as the 9-β-L-adenosine generated from AMP) which trigger signal transduction pathways that lead to transcriptional activation of certain genes, to regulation of specific enzyme activities (e.g. of Rubisco in the photosynthetic Calvin cycle and of nitrate reductase in the nitrogen assimilation) and membrane transporters. All these lead to stimulation of growth and developmental processes, both under normal and stress conditions. Applied as a foliar spray or added to the aqueous nutrient solution in concentrations as low as 10^{-8}-10^{-6} M, Tria could efficiently counteract the deleterious effects of high salinity, drought, extreme temperatures, hypoxia, high light intensity, heavy metal pollution, UV-B radiation and other environmental stressors on several physiological processes in various crop plants (Islam and Mohammad, 2020; Zaid et al., 2020; Tompa et al., 2022). In different medicinal and aromatic plants, external application of micromolar amounts of Tria led to an increased production of alkaloids and essential oil-constituting monoterpenes (Naeem et al., 2012).

Cytokinins are plant hormones with well-known developmental and metabolic effects, being mostly responsible for stimulation of cell divisions in meristematic regions (root tip, shoot apex, embryo of the seed), for the delay of senescence due to maintenance of a metabolically active state of plant organs, for the development of thylakoid membrane system in the chloroplasts, as well as for acclimation mechanisms to drought, high salinity, chilling and heat stress (Haisel et al., 2006; El-Ghamery and Mousa, 2017; Li et al., 2020). Because it is less expensive than the pure form of the natural cytokinins, the synthetic cytokinin benzyladenine (BA, also known as 6-benzyl-aminopurine) is the most widely used one in the *in vitro* plant tissue and organ cultures, mainly to induce callus formation and generation of new shoots, in co-action with different auxins. Upon the perception of cytokinins, hystidine kinases located in the plasma membrane or in the membrane of the endoplasmic reticulum initiate intracellular signaling pathways which will regulate the activity of several genes, resulting in coordinated developmental changes (Hwang et al., 2012; Rademacher, 2015). Although many effects of cytokinins in plants were demonstrated under various growth conditions, their application in horticulture, in

ex vitro cultivation of medicinal plants and in human therapy is still very limited.

St. John's wort (*Hypericum perforatum* L.) is one of the most popular medicinal plants, being an excellent example for how modern phytochemical research can be effective in the development of traditional remedies (Nahrstedt and Butterweck, 2010). It is mainly used to treat mild and moderate forms of depression, having similar effectiveness but fewer side effects than standard synthetic antidepressants. This effect is related to the fact that its certain active compounds act on noradrenergic, dopaminergic and serotonergic systems in the human brain. The main active secondary metabolites responsible for these effects are the hypericins (naphthodianthrones, which include hypericin, isohypericin, protohypericin and pseudohypericin) and the hyperforins (prenylated phloroglucinols represented by hyperforin, adhyperforin and their derivatives). These components are also used to treat anxiety and certain types of insomnia. Other, more common active metabolites are phenolic compounds such as the flavonoids quercetin (with high antioxidant capacity), biapigenin and kaempferol, as well as chlorogenic acid and caffeic acid (Kasper et al., 2012; Russo et al., 2014; Shrivastava and Dwivedi, 2015). Oil-based extracts of the herb are used externally to treat wounds, burns, swellings and skin bruises. It was demonstrated that hyperforins strengthen the skin barrier function by reducing harmful radical formation, and they are also responsible for the activation of cytochrome P450-metabolizing enzymes, while hypericins inhibit the enzyme protein kinase C and the release of arachidonic acid, thus having an anti-inflammatory effect. The skin healing effect is also related to the fact that extracts of St. John's wort stimulate collagen synthesis and fibroblast migration (Edwards et al., 2015). Recently it was established that the plant's extracts possess antibacterial effects, including against mycobacteria, and they are also efficient against certain types of viruses, such as the influenza virus and retroviruses (Avato et al., 2004; Mortensen et al., 2012). Furthermore, hypericins and hyperforins were effective in the *in vitro* inhibition of tumor cell divisions and in the induction of apoptotic cell death in lymphomas (Shrivastava and Dwivedi, 2015).

The aim of this work is to enhance growth and photosynthetic performance of young St. John's wort plants with the foliar application of micromolar concentrations of triacontanol and benzyladenine, as well as to study the possible interactions between the effects of the above-mentioned bioactive compounds when they are applied simultaneously. The starting prediction is that if Tria and BA induce different signal transduction pathways in triggering certain metabolic and developmental processes, their effects may be synergistic or additive, resulting in a more pronounced growth stimulation than in the case of their separate application. Our results may contribute to optimization of biomass production of this medicinal plant during cultivation, this being a prerequisite for an enhanced yield of herbal medicinal product.

2. Materials and methods

Plant material and experimental conditions

Seeds of St. John's wort (*Hypericum perforatum* L.) were purchased from Agrosel S.R.L., soaked for 24 h in dechlorinated tap water and sown individually in round pots (9.0 cm diameter and 11.8 cm height) filled with wet perlite as an inert soil substitute. The pots were randomly arranged in a growth chamber, where the temperature was kept constantly at 22 °C, the relative air humidity was set to 60%, and the photosynthetically active photon flux density, provided by blue and red LEDs, was 355 µM m^{-2} s^{-1} for a daily 14 h photoperiod.

After germination, the plantlets were watered every three days with Hoaglans's mineral nutrient solution, which provided in an optimal molar ratio every essential macro-, micro- and ultramicronutrient, having the pH value set to 5.6 (Hoagland and Arnon, 1950). Treatments with bioactive compounds started when plantlets were six days old and lasted for 15 days, being repeated once in three days, during the morning hours. Thus, each plant received a total number of five treatments during the experiment. For every experimental variant seven similar plantlets were selected, representing a total of 28 plants in separate pots. Control plants were not treated with any biostimulant, one set of plantlets received once in three days, as a foliar spray, 1 µM triacontanol (Tria, purchased from Nutri-Tech Solutions, Yandina, Australia), another group of plants was treated with the same periodicity with 2 µM benzyladenine (BA, 6-benzyl-aminopurine, provided by Sigma-Aldrich, Darmstadt, Germany). Another set of seven plantlets was provided simultaneously, as foliar spray, with 1 µM Tria and 2 µM BA. The solvent of the Tria and BA solutions was distilled water. The concentrations were chosen based on previous sets of experiments and on literature (El-Ghamery and Mousa, 2017; Tompa and Fodorpataki, 2021). Plants were rerandomized after each treatment event to avoid any positional influence.

Growth and yield measurements

During germination of seeds, parameters such as germination percentage, rate of germination, mean germination time and germination speed were recorded or computed. After germination, plant shoot height (from the collet to the stem apex) was measured on a daily basis, for seven plants for each experimental variant. On the fifteenth day after the initiation of treatments (i.e. 21 days after the beginning of germination), after performing the *in vivo* and *in situ* measurment of induced chlorophyll fluorescence parameters, the fresh biomass of the aboveground shoot and the fresh weight of leaves (including the young ones emerging from the apical bud and possessing their own petiole) of all plantlets were determined separately, using an analytical scale.

Determination of induced chlorophyll fluorescence parameters

Pulse amplification modulated and conventional parameters of chlorophyll fluorescence were determined with an FMS-2 type fluorometer (Hansatech, Norfolk, UK) on the adaxial side of the third fully developed leaf blade from the base of the stem. Conventional parameters, such as ground fluorescence level (Fo), maximal fluorescence (Fm), fluorescence variation (Fv = Fm – Fo) and potential quantum yield of photosystem II (Fv/Fm) were determined in leaves that were dark-adapted for 15 min before measurements, while light pulse amplification modulated parameters (the Fo' modulated ground fluorescence, the Fm' modulated maximal fluorescence, the Fs steady-state fluorescence value and Φ, the effective quantum yield of photosystem II, calculated with the relation (Fm' – Fs) / Fm') were established in leaves exposed to ambient illumination (Haisel et al., 2006; Tompa et al., 2022). The vitality index of the photosynthetic apparatus of thylakoid membranes involved in the light reactions of photosynthesis, expressed as the chlorophyll fluorescence decrease ratio [Rfd = (Fm – Fs)/Fs], was determined according to Lichtenthaler et al. (2005), applying very strong white light (with the intensity of 3000 µmole photons m^{-2} s^{-1}) for five minutes.

Determination of photosynthetic pigment content of leaves

For the measurement of photosynthetic pigment content (chlorophyll *a*, chlorophyll *b* and total carotenoids, i. e. carotenes and xanthophylls) the same leaves were used as the ones on which the *in vivo* determination of induced chlorophyll fluorescence was performed previously (from seven plants for each experimental variant). The leaf blades were used for extraction of chlorophylls and carotenoids after the fresh weight of shoots and leaves was measured. 0.2 g of leaves were ground in a prechilled mortar with 5 mL 80% (v/v) acetone, in the presence of a small amount of magnesium carbonate, to avoid transformation of chlorophylls into phaeophytin. The acetonic extracts were centrifuged for 10 min at 4000 g and 4 °C. The absorbance of 4 mL of supernatant was measured with a spectrophotometer (V-750 UV-Vis Jasco, Midrand, South Africa) at 470 nm, 646 nm and 663 nm, using as reference the 80% acetone (Wellburn, 1994). The chlorophyll and carotenoid pigment contents were expressed as mg per gramm fresh weight of leaves, using the formulae: chlorophyll *a* content = (12.21 x A663 – 2.81 x A646) x V x D / 1000 x d x W, chlorophyll *b* content = (20.13 x A646 – 5.03 x A663) x V x D / 1000 x d x W, carotenoids content = (A470 – 3.27 x chlorophyll *a* content – 104 x chlorophyll *b* content) x V x d / W x d, where A is the absorbance of pigment extract at the given wavelength, V is the volume of the acetonic leaf extract (in mL), D is the coefficient of dilution, d is the width of the measuring cuvette through which the light beam crosses the pigment solution (in cm), and W is the fresh weight of leaf blade used for the extraction (in g).

Statistical analysis of experimental data

Data analysis was performed using the R statistical package (R Core Team, software environment version 4.1.0). Normality of experimental data distribution was tested with the Shapiro-Wilk test, while Bartlett's test was applied for establishing the homogeneity of variances. Significance of differences between experimental variants was determined with one-way ANOVA, followed by the Tukey HSD multiple means comparison test. Every experimental variant had 7 replicates, and every measurement was performed with two technical repetitions (i. e. every measurement was repeated twice). The data were represented as mean ± standard error, and differences were interpreted as significant at $p < 0.05$.

3. Results and discussions

Influences on vegetative growth and development

21 days after germination, the shoot elongation of young plantlets was stimulated in the same degree by triacontanol and by benzyladenine, but the combination of the two bioactive compounds did not result in further enhancement of internode elongation (**Fig. 1**). This reflects that there is no interaction (neither synergism, nor antagonism) between triacontanol and benzyladenine in the stimulation of stem length. With all the three types of treatments, an increment of about 50% could be recorded in the shoot height as compared to the untreated control, which represents a significant growth stimulation and enables the plantlets to develop higher stems in shorter times, which may facilitate a better tolerance to adverse conditions, thus increasing the survival expectations of the vulnerable young plantlets. Similar results were reported for onion plantlets, where BA treatment stimulated root

and shoot length (El-Ghamery and Mousa, 2017), and for spinach, where even smaller concentrations of Tria (25 nM) significantly increased shoot height (Tompa and Fodorpataki, 2021). The combined treatment did not result in any significant difference from the separate treatments with Tria and BA, respectively. While no synergism could be demonstrated between Tria and BA in the stimulation of stem elongation, the fresh biomass of the vegetative shoots (stems with leaves) was significantly higher when the two bioactive substances were provided together, as compared with the control and with the separate treatments with Tria and with BA, respectively (**Fig. 2**).

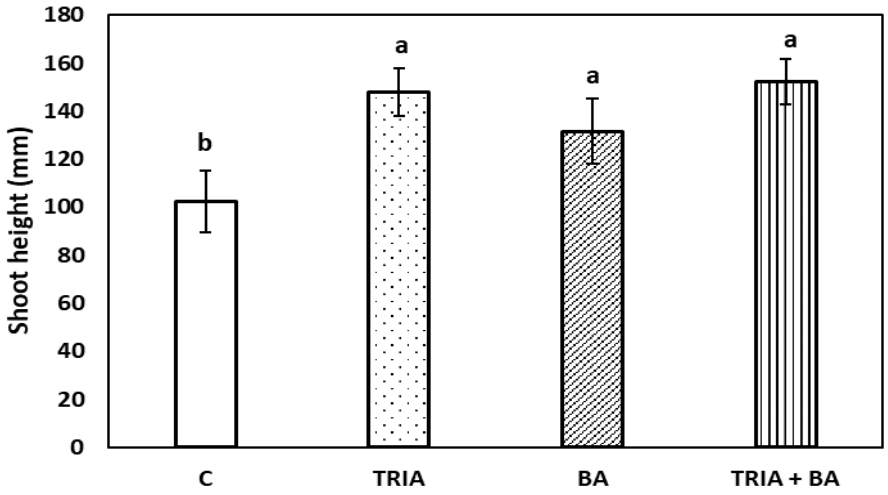

Fig. 1. Stimulation of shoot elongation by exogenously applied 1 µM triacontanol (TRIA), 2 µM benzyladenine (BA) and their combination (TRIA + BA) in 21 days old St. John's wort plantlets. C – control group. Vertical bars show the ± standard errors from means (n = 7), and the different letters represent significant differences at $p < 0.05$

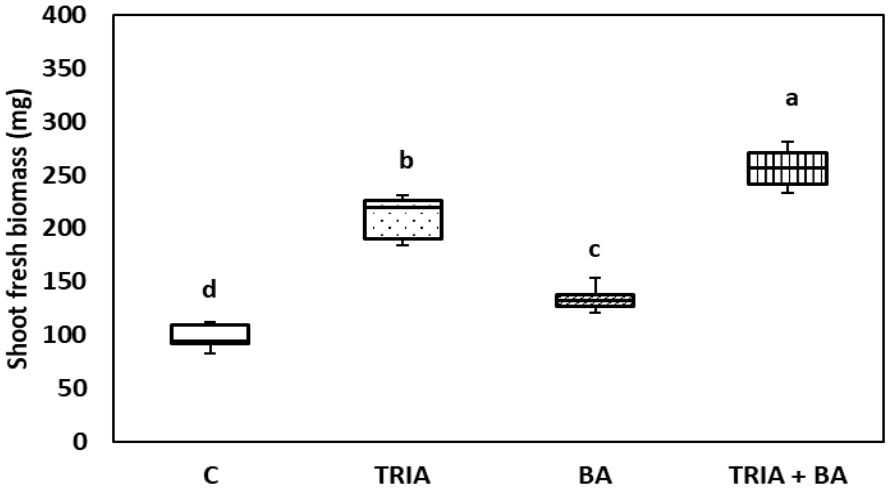

Fig. 2. Influence of 1 µM triacontanol (TRIA), 2 µM benzyladenine (BA) and their combination (TRIA + BA) on shoot fresh biomass accumulation of 21 days old St. John's wort plantlets. C – control group. Vertical bars show the ± standard errors from means (n = 7), and the different letters represent significant differences at $p < 0.05$

This reveals a positive interaction between Tria and BA in stimulating fresh weight accumulation of young St. John's wort plantlets, because none of the two growth regulators could induce such an increment by its own, at least in the applied concentrations and for the 15 days period of treatments. Applied individually, Tria and AB still enhanced in a statistically significant degree the net biomass production of plants, the stimulating effect of triacontanol being more pronounced than the one exerted by benzyl-adenine.

Increased aboveground vegetative biomass growth induced with Tria was also reported for spinach plants, the same degree of stimulation being achieved with 25 nM and 1 µM concentrations (Tompa et al., 2022). For BA, the highest shoot number was obtained when 2 mg L^{-1} BA was added to the nutrient medium of *in vitro* shoot cultures of St. John's wort, while the highest hypericin percentage in the fresh biomass was achieved when 1 mg L^{-1} BA was supplied exogenously (Karakas et al., 2009). It is worth mentioning that the plantlets produce their own quantity of cytokinins, and the added benzyladenine is an extra amount which increases the overall cytokinin content, also modifying the molar ratio between the different growth regulators, thus leading to further changes in growth and development (Li et al., 2020).

Because the highest amounts of hypericins, hyperforins and other pharmacologically active secondary metabolites accumulate in leaves, especially in the large intercellular spaces that develop in the mesophyll (Shrivastava and Dwivedi, 2015), the influence of the two growth regulators on the total leaf biomass of each plant was also evaluated (**Fig. 3**). Tria did not cause a significant increase in the fresh biomass of leaves as compared to the control group, the exogenous application of 2 µM BA as foliar spray caused a moderate, but statistically significant decrement in the leaf biomass value per plant, and the only increment of this growth parameter was obtained when a combined treatment was applied with Tria and BA.

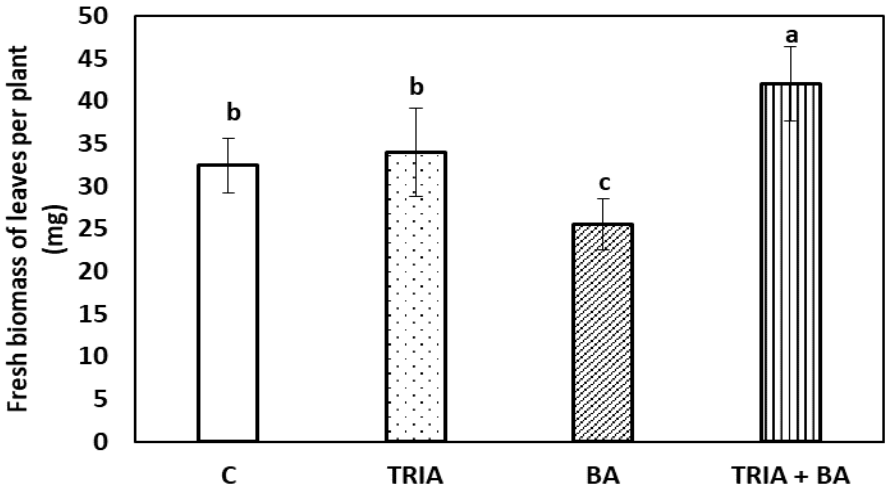

Fig. 3. Influence of 1 µM triacontanol (TRIA), 2 µM benzyladenine (BA) and their combination (TRIA + BA) on the fresh biomass of leaves of 21 days old St. John's wort plantlets. C – control group. Vertical bars show the ± standard errors from means (n = 7), and the different letters represent significant differences at $p < 0.05$

This means that there is such an interaction between Tria and BA in the plants that while none of the compounds increases the fresh weight of leaves when applied separately, their combination results in a significant stimulation of leaf growth. The internal mechanisms leading to this interaction still need to be elucidated with further investigations.

Influences on photosynthetic light use efficiency

The overall vitality of plants, their biomass production and the capacity to produce different amounts of metabolic products highly depend on their photosynthetic performace. This can be evaluated non-destructively by registering different parameters of the induced chlorophyll fluorescence in leaves. These parameters give an insight into the energetics of light reactions, rendering possible to evaluate the efficiency of photochemical conversion of the light energy absorbed by the photosynthetic pigments, in order to be stored and incorporated in the new organic compounds built-up by carbon dioxide assimilation (Lichtenthaler et al., 2005). A valuable parameter of the photosynthetic performance is the effective quantum yield of photosystem II (Φ), which correlates with the proportion of the absorbed light energy that under the given growth conditions can be used after conversion in the production of new metabolites. When St. John's wort plantlets were treated separately with Tria and BA, in both cases a moderate, but statistically significant increase of the effective quantum yield could be registered, while its highest value (approaching 0.8) was reached in plants that have received both growth regulators simultaneously (**Fig. 4**). This is one more evidence for the synergism between Tria and BA in stimulating metabolic processes, such as photosynthetic light conversion into chemical energy. The capacity of Tria to enhance photosynthetic processes, especially under environmental stress conditions which impair the photochemical reactions or modify the chemical composition of thylakoid membranes in chloroplasts, was also demonstrated in certain crop plant species (Godoy et al., 2021; Tompa et al., 2022).

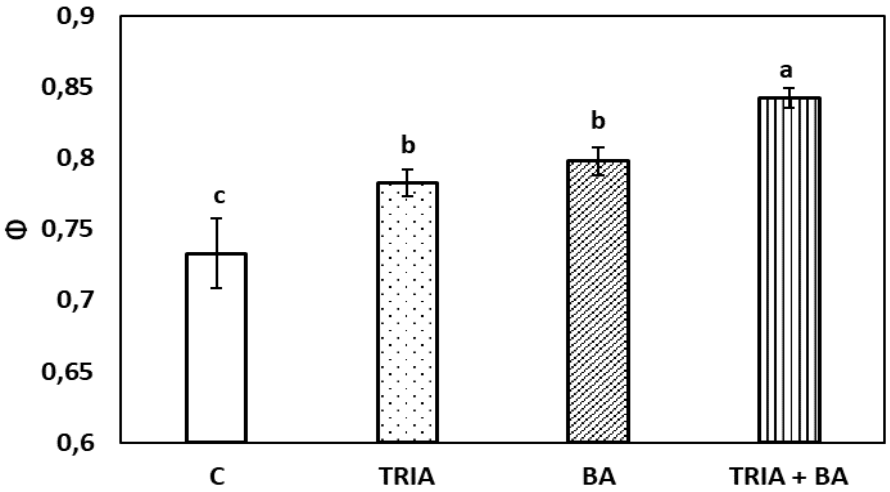

Fig. 4. Influence of 1 µM triacontanol (TRIA), 2 µM benzyladenine (BA) and their combination (TRIA + BA) on the effective quantum yield of photosystem II (Φ) in leaves of St. John's wort plantlets. C – control group. Vertical bars show the ± standard errors from means (n = 7), and the different letters represent significant differences at $p < 0.05$

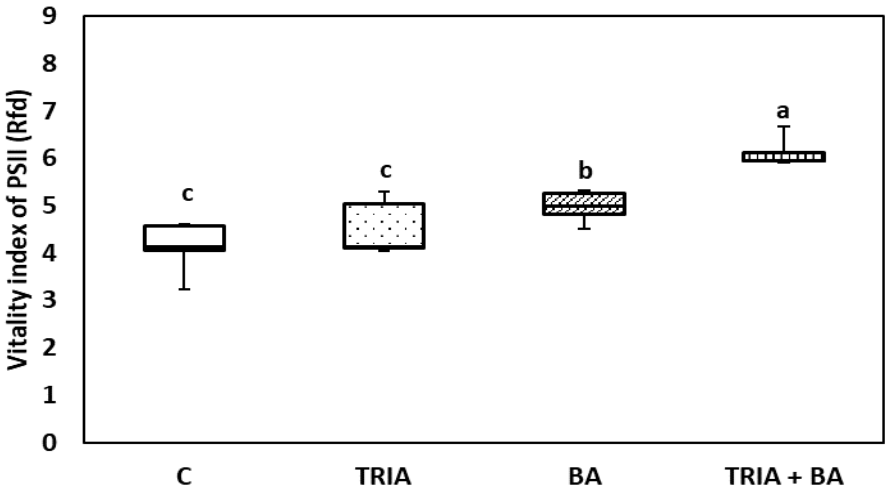

Fig. 5. Vitality index of the photosynthetic apparatus in leaves of St. John's wort plantlets treated with 1 µM triacontanol (TRIA), 2 µM benzyladenine (BA) and their combination (TRIA + BA). C – control group; PSII – photosystem II; Rfd – relative fluorescence decrease, obtained from measurements of induced chlorophyll fluorescence. Vertical bars show the ± standard errors from means (n = 7), and the different letters represent significant differences at $p < 0.05$

For BA there is also evidence for its beneficial influence on the development of the photosynthetic apparatus of leaves (Haisel et al., 2006), but we could not find any evidence on the possible interrelations between Tria and BA concerning any step of the photosynthetic process.

Under certain conditions (prolongued illumination of leaves with oversaturating white light beams), parameters of the induced chlorophyll fluorescence may be used to evaluate the overall functionality of the photosynthetic apparatus under the given developmental conditions (Lichtenthaler et al., 2005), with the determination of a physiological marker known as the vitality index of photosystem II (PSII), expressed as the relative fluorescence decrease (Rfd). When St. John's wort plants were treated separately with Tria and BA, only BA led to a significant increase in the vitality index of PSII, but when Tria was combined with BA, this increment was even more pronounced (Rfd increased from about 4 in the control group to 5 under the influence of BA and further to the value of 6 in the leaves of plants receiving both BA and Tria, **Fig. 5**). This is another parameter that demonstrates the existence of an interaction between Tria and BA in the up-regulation of photosynthetic processes, in this case Tria further enhances the stimulating capacity of BA.

Influences on carotenoid pigment content

Changes in the photosynthetic pigment content of leaves are usually reliable biochemical markers of many external influences exerted on plant metabolism. Treatments with Tria and BA did not cause any significant change in the chlorophyll *a* and chlorophyll *b* content of St. John's wort leaves (their interval remaining around 1.67 ± 0.24 mg g^{-1} for chlorophyll *a* and 0.56 ± 0.04 mg g^{-1} for chlorophyll *b*, data not shown), but the carotenoid pigment content was increased with the same degree by all of the three types of treatments (with Tria, with BA and with their combination).

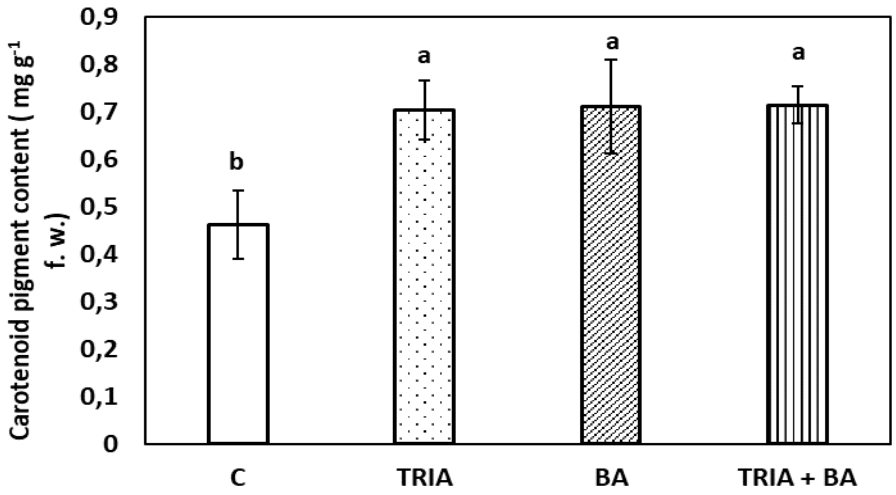

Fig. 6. Influence of 1 µM triacontanol (TRIA), 2 µM benzyladenine (BA) and their combination (TRIA + BA) on the carotenoid pigment content in leaves of St. John's wort plantlets. C – control group; f.w. – fresh weight. Vertical bars show the ± standard errors from means (n = 7), and the different letters represent significant differences at $p < 0.05$

When applied together, Tria and BA did not lead to a more increased carotenoid content than in the case when they were applied separately on different plants, indicating that these two bioactive compounds do not interact in the metabolism of carotenes and xantho-phylls (**Fig. 6**). The increment of carotenoid pigment content could be beneficial under very high photon flux densities and under several other environmental stress conditions which induce photooxidative damage in the photo-synthetic apparatus. It was demonstrated that carotenoids act as valuable non-enzymatic antioxidants by protecting the photosynthetic membranes from severe damages caused by over-generation of singlet oxygen and other reactive oxygen species (Garcia-Garcia et al., 2020). In experiments conducted with spinach, 1 µM Tria did not induce an increment in the carotenoid pigment content of leaves, but managed to restore the original carotenoid content in plants exposed to salt stress generated with 150 mM sodium chloride (Tompa et al., 2022). In the case of BA, experiments with bean, tobacco, sugar beet and maize plantlets showed that 10 µM BA could increase the chlorophyll pigment content of chloroplasts when plants were exposed to drought stress. The increment caused by water deficiency in the content of the carotenoids implied in the protective xanthophyll cycle was further enhanced when plantlets were treated with BA, demonstrating its implication in the stimulation of the protective capacity of carotenoids against photooxidative damages (Haisel et al., 2006).

Conclusions

Interaction between exogenously applied triacontanol and benzyladenine enhances photo-synthetic performance and biomass production of St. John's wort plantlets, beyond the stimulation exerted separately by the two growth regulators. Shoot height and carotenoid pigment content of leaves are also increased both by 1 µM Tria and 2 µM BA, but the combined treatment with these compounds does not lead to further stimulation of stem elongation and accumulation of carotenoids. Synergism of Tria and BA in the up-regulation of specific metabolic and developmental processes may be exploited to optimize the

cultivation of this medicinal plant and this may be a prerequisite to increase the content of active compounds in the herbal product. For this later purpose further investigations are needed concerning the influence of different concentrations of triacontanol and benzyl-adenine, applied separately or together, on more physiological processes that are relevant for the secondary metabolic pathways involved in the biosynthesis of the desired pharmaco-logically active 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.

References

1. Avato P, Raffo F, Guglielmi G, Vitali C, Rosato A (2004) Extracts from St. John's wort and their antimicrobial activity. Phytother Res 18(3):230–232. doi: 10. 1002/ptr.1430
2. Edwards SE, Rocha IC, Williamson EM, Heinrich M (2015) Phytopharmacy: an evidence-based guide to herbal medicinal products. John Wiley & Sons Ltd, Singapore, pp 335–339
3. El-Ghamery AA, Mousa MA (2017) Investigation on the effect of benzyladenine on the germination, radicle growth and meristematic cells of *Nigella sativa* L. and *Allium cepa* L. Ann Agric Sci 62(1):11–21. doi: 10.1016/j.aoas.2016.11.002
4. Garcia-Garcia AL, Garcia-Machado FJ, Borges AA, Morales-Sierra S, Boto A, Jimenez-Arias D (2020) Pure organic active compounds against abiotic stress: a biostimulant overview. Front Plant Sci 11:575829. doi: 10.3389/fpls.2020.575829
5. Godoy F, Olivos-Hernandez K, Stange C, Handford M (2021) Abiotic stress in crop species: improving tolerance by applying plant metabolites. Plants 10:186–195. doi: 10.3390/ plants10020186
6. Haisel D, Pospisilova J, Synkova H, Schnablova R, Batkova P (2006) Effects of abscisic acid or benzyladenine on pigment contents, chlorophyll fluorescence and chloroplast ultrastructure during water stress and after rehydration. Photosyn-thetica 44(4):606–614. doi: 10.1007/ s11099-006-0079-5
7. Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil. Calif Agric Exp Stat Circ 347:1–32
8. Hwang I, Sheen J, Muller B (2012) Cytokinin signaling networks. Annu Rev Plant Biol 63: 353–380. doi: 10.1146/ annurev-arplant-042811-105503
9. Islam S, Mohammad F (2020) Triacontanol as a dynamic growth regulator for plants under diverse environmental conditions. Physiol Mol Biol Plants 26(5):871–883. doi: 10.1007/ s12298-020-00815-0
10. Karakas O, Toker Z, Tilkat E, Ozen HC, Onay A (2009) Effects of different concentrations of benzylaminopurine on shoot regeneration and hypericin content in *Hypericum triquetrifolium* Turra. Nat Prod Res 23(16):1459–1465. doi: 10.1080/ 14786410701664528
11. Kasper S, Caraci F, Forti B, Drago F, Aguglia E (2012) Efficacy and tolerability of Hypericum extract for the treatment of mild to moderate depression. Eur Neuropsych 20(11):747–756. doi: 10.1016/ j.euroneuro.2010.07.005
12. Li S-M, Zheng H-X, Zhang X-S, Sui N (2021) Cytokinins as central regulators during plant growth and stress response. Plant Cell Rep 40:271–282. doi: 10.1007/ s00299-020-02612-1

13. Lichtenthaler HK, Buschmann C, Knapp M (2005) How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll fluorescence decrease ratio Rfd of leaves with PAM fluorometer. Photosynthetica 43:379–393. doi: 10.1007/s11099-005-0062-6
14. Mortensen T, Shen S, Shen F, Walsh MK, Sims RC, Miller CD (2012) Investigating the effectiveness of St. John's wort herb as an antimicrobial agent against mycobacteria. Phytother Res 26(9):1327–1333. doi: 10.1002/ptr.3716
15. Naeem M, Masroor M, Khan A, Moinuddin M (2012) Triacontanol: a potent growth regulator in agriculture. J Plant Interact 7(2):129–142. doi: 10.1080/17429145.2011. 619281
16. Nahrstedt A, Butterweck V (2010) Lessons learned from herbal medicinal products: the example of St. John's wort. J Nat Prod 73(5):1015–1021. doi: 10.1021/np.1000329
17. Oshchepkov MS, Kalistratova AV, Savelieva EM, Romanov GA, Bystrova NA, Kochetkov KA (2020) Natural and synthetic cytokinins and their applications in biotechnology, agrochemistry and medicine. Russ Chem Rev 89(8):787-810. doi: 10.1070/RCR4921
18. Rademacher W (2015) Plant growth regulators: background and uses in plant production. J Plant Growth Regul 34:845–872. doi: 10.1007/s00344-015-9541-6
19. Russo E, Scicchitano F, Whalley BJ, Mazzitello C, Ciriaco M, Esposito S, Patane M, Upton R, Pugliese M, Chimirri S, Mammi M, Palleria C, De Sarro G (2014) *Hypericum perforatum*: pharmacokinetics, mechanism of action, tolerability and clinical drug-drug interactions. Phytother Res 28(5):643–655. doi: 10. 1002/ptr.5050
20. Shrivastava M, Dwivedi LK (2015) Therapeutic potential of *Hypericum perforatum*: a review. Intl J Pharmac Sci Res 6(12):1000–1007. doi: 10.13040/IJPSR. 0975-8232.6(12). 1000.07
21. Tompa B, Fodorpataki L (2021) Influence of triacontanol and salt stress on the growth and metabolism of spinach. Acta Univ Sap Agric Environ 13:65–76. doi: 10.2478/ ausae-2021- 0006
22. Tompa B, Balint J, Fodorpataki L (2022) Enhancement of biomass production, salinity tolerance and nutraceutical content of spinach (*Spinacia oleracea* L.) with the cuticular wax constituent triacontanol. J Appl Bot Food Qual 95:121–128. doi: 10.5073/JABFQ.2022. 095.00X
23. Tsukagoshi S, Yamori W (2020) Beneficial effects of various environmental stresses on vegetables and medicinal plants for the production of high value-added plants. In: Pessarakli M (ed) Handbook of plant and crop stress. CRC Press, Boca Raton, USA, pp 909–918
24. Wellburn AR (1994) The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J Plant Physiol 144:307–313. doi: 10.1016/S0176-1617-81192-2
25. Zaid A, Asgher M, Wani IA, Wani SH (2020) Role of triacontanol in overcoming environmental stresses. In: Roychoudhury A, Tripathi DK (eds) Protective chemical agents in the amelioration of plant abiotic stress. John Wiley & Sons Ltd, Croydon, UK, pp 491–509.

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BIODEGRADATION OF ANTHRACENE AND PHENANTHRENE BY *PSEUDOMONAS STUTZERI* (BUK_BTEG1) ISOLATED FROM PETROCHEMICAL CONTAMINATED SOIL

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Abstract: The United States Environmental Protection Agency (USEPA) has identified 16 substances as priority polycyclic aromatic hydrocarbons (PAHs) that are harmful to humans, including anthracene and phenanthrene. These substances are pervasive pollutants introduced into the environment through anthropogenic and natural processes, causing ecological concerns and necessitating the quest for new strains capable of biodegrading these toxins. A novel strain of the genus *Pseudomonas* was isolated and molecularly identified based on partial 16S rRNA and phylogenetic analysis as *Pseudomonas stutzeri* strain BUK_BTEG1 from petrochemical contaminated soil. One factor at a time (OFAT) in Bushnell-Haas (BH) media was used to optimize the strain's biodegradation conditions. The isolate could grow up to 600 mgL^{-1} and 400 mgL^{-1} of anthracene and phenanthrene as the sole carbon source at an optimum pH of 7.0 and 7.5 respectively, inoculum concentration of 4% (v/v), and temperature of 35°C during 72 hours of incubation. The strain could degrade phenanthrene and anthracene to a maximum of 99 and 72 percent, respectively, under ideal conditions. The breakdown products' GC-MS analysis revealed the existence of the pathway's main metabolites, catechol, salicylic acid, and derivatives of phthalic acid. The strain exhibits promising potential for use in the bio-cleansing of environments contaminated by PAHs.metabolites.

Keywords: polycyclic aromatic hydrocarbons (PAHs), biodegradation, anthracene, phenanthrene, bioremediation

1. Introduction

Over time, the understanding of the potential negative effects of pollutants on the environment and public health has grown on a global scale (Ghosal et al., 2016). Anthracene is one of the hazardous chemicals, such as polycyclic aromatic hydrocarbons (PAHs), that are deposited and accumulated in soils due to ongoing pollution with crude oil and its derivatives (Gupte et al., 2016). Organic molecules known as PAHs are made up of two or more fused aromatic rings. The burning of organic materials produces very few of these chemicals. However, since the start of the industrial revolution powered by fossil fuels, they have multiplied in number due to careless human behavior (Chowdhury et al., 2017). Petroleum contamination occurs as a result of above- and below-ground storage tank leaks,

petroleum product spills during transportation, abandoned manufacturing sites for gasoline, other unintentional discharges, and ongoing industrial activities (Nzilaet al., 2018). Petroleum is a source of toxins that can harm the health of plants, animals, and people because it contains risky chemicals including anthracene and phenanthrene, among other PAHs. Exposure to PAHs has been linked to substantial toxicological hazards and has been shown to be genotoxic, mutagenic, and carcinogenic in nature (Fritt-Rasmussen et al., 2015; White et al., 2016; Ibrahimet al., 2018). The US Environmental Protection Agency regarded sixteen PAHs as toxic to humans. Anthracene and its iso-forms have been classified among those sixteen toxic priority PAHs (Zelinkova and Wenzl, 2015). The risks associated with PAH exposure highlight the significance of a comprehensive remediation approach for a PAH-polluted environment (Haritash and Kaushik, 2009).

Bioremediation has grown in popularity in recent decades as a cost-effective, viable, and safe method of cleaning up contaminated places. It makes use of microorganisms, which can feed on dangerous toxins such as PAHs and create innocuous molecules as a result (Ghosal et al., 2016; Oaikhenaet al., 2019; Rabiu and Gimba, 2021). From the beginning of the last decade to date, a number of bacteria strains have been identified as "PAH Degraders" including the common genera of *Pseudomonas, Proteus, Rhodococcus, Stenotrophomonas*, *Bacillus, Alcaligenes,* and *Mycobacterium* (Singh and Tiwary 2016; Ibrahim et al., 2018; Salamat et al., 2018; Suzuki and Takizawa, 2019; Elufisanet al., 2020; Shehu et al., 2021).

Although two basic multistep mechanisms for bacteria to break down PAHs have been reported, depending on whether the process requires oxygen (aerobic) or does not require oxygen (anaerobic) (Ghosal et al., 2016; Li et al., 2021). However, given favourable conditions, the aerobic degradation pathway has been found to be the relevant route in surface layer soils, which contains oxygen and is dependent on gene products, typically via the activity of enzymes such as oxygenases, peroxidases, and hydratases (Ghosal et al., 2016; Gupte et al., 2016)

The persistent nature of these pollutants in the environments coupled with the health problems posed by PAHs and their metabolites, it is, however, important to search for native PAH degrading strains from a microbial community in the natural environment capable of bio-cleaning the environment. The present study focused on the isolation, identification, and characterization of anthracene and phenanthrene degrading bacterium from petrochemical-contaminated soil.

2. Materials and methods

Sample collection

A soil sample was taken from Kwakwachi Mechanic village located in DawakinTofa LGA of Kano State with GPS coordinate N 12°1'18'' E8°31'28''. The sample was taken from the soil's surface to a depth of 15 cm, evenly mixed, and carefully placed in a sterile polyethylene bag stored at room temperature before being transferred to the laboratory for bacteria isolation.

Enrichment and isolation of PAH-degrading bacteria

Enrichment and isolation of anthracene and phenanthrene degrading strain BUK_BTEG1 was carried out using a modified method of Patel et al. (2018), the strain was enriched in Bushnell-Haas (BH) liquid medium containing 1000mgL^{-1} anthracene and phenanthrene (as the sole carbonsource) and

incubated aerobically at 37°C. The medium is made up of 1gNH_4NO_3, 0.02 g $CaCl_2$, 0.2 g $MgSO_4$, 1 g KH_2PO_4, 1 g K_2HPO_4, and 0.05 g of $FeCl_3$ in 100 mL of distilled water. In the instance of Bushnell Hass Agar, 15 g of agar-agar was added and autoclaved. To begin the enrichment, 10 g of sediment was suspended in a 250-mL Erlenmeyer flask with 100 mL BH broth containing 500 mgL^{-1} of each particular PAH as the only carbon and energy source. For 7 days, the strainswere cultured at 37°C in an incubator shaker set to 120 rpm. This procedure was performed several times. Following four successive cultivations, the bacteria were inoculated on BH agar supplemented with 500mgL^{-1} anthracene and phenanthrene as the only carbon and energy source separately using the conventional surface spray-plate technique and incubated at 37°C for 72 hours to examine bacterium growth. The isolate formed was kept for identification and characterization.

Extraction of genomic DNA and PCR amplification

A loopful of the isolate was inoculated in 5 mL Laurie-Bertani (LB) medium and incubated at 37°C for 24 h. The bacteria suspension formed was centrifuged at 10,000 for 5 min, then the DNA was extracted following the protocol described by Neerajaet al. (2013) and Ya'uet al. (2020). The PCR for the amplification of the 1.5 kb of the partial segment of the 16S rRNA reaction was carried out using KAPATaq DNA polymerase. The total reaction volume was 25 µL. The reaction mixture comprises 2 µL each of the genomic DNA, 2.5 µL of 10 TaqA Buffer, ~0.4 M (0.85 µL) of each of forward 5-*TGGAGAGTTTGATCCTGGCTCAG*-'3 and reverse primer 5'-*TACCGCGGCTGCTGGCAC* -3' from Sigma-Aldrich, United Kingdom, were used (Kumar et al., 2018). 1.5 µL of $MgCl_2$ (1.25mM), 0.2 µL of dNTP (0.25 mM) and 0.2 µL of Taq DNA polymerase made up to 25 µL with ddH_2O. The following conditions were used for the PCR amplification: initial denaturation of 5 min at 95°C, 30s at 60°C of primer annealing and 1 min at 72°C extensions then followed by 10 min of final extension at 72°C (Kumar et al., 2018).The size of the DNA fragment was compared with the Hyper Ladder-2K marker on a 1.5% agarose gel (CSL-AG500, Cleaver Scientific Ltd) and observed using Ingenius' Syngene Gel Documentation System after the last elongation cycle (IG31459). The presence of a product of the predicted size was seen as a favorable outcome.

Sequencing and phylogenetic analysis

The gel extraction kit QIA fast Qiagen was used to extract the 16S rRNA PCR product from the gel (Promega, USA). DNA sequencing was performed according to the manufacturer's instructions using the ABI Prism Big DyeTM 3730/3730XL Terminator Cycle Sequencing Ready Reaction Kit. The sequence was then searched in the nucleotide databases of the National Center for Biotechnology Information (NCBI) to identify the organism. The NCBI/GenBank 16S rRNA gene sequences of type strains of *Pseudomonas* species were obtained, and sequence alignments were performed using the software ClustalW, followed by the building of a neighbor-joining phylogenetic tree. Molecular Evolutionary Genetics Analysis (MEGA 11.0) for Windows was used to compute the evolutionary connection using the Maximum Composite Likelihood technique (Tamura et al., 2021). The sequence was submitted to the NCBI GenBank with the entry number OM039162.

Characterization of anthracene and phenanthrene degradation

Anthracene and phenanthrene degradation by the strain was studied using the One-Factor-at-a time (OFAT) approach by optimizing the growth conditions. The parameters optimized were nitrogen source and concentration, pH, initial substrate (Anth and Phen) concentration, and temperature. In 100 mL BH medium, each parameter was tested progressively while keeping the previously optimized values in mind in 100 mL BH media on an orbital shaker (150 rpm) at 37°C (the temperature was optimized separately) for a period of 120h. The incubation time was examined from 24 h – 120 h. Ammonium chloride, ammonium nitrate, ammonium sulfate, and sodium nitrate were the nitrogen sources used. The best was ammonium nitrate, which was then evaluated at concentrations ranging from 200 mgL^{-1}-1400 mgL^{-1} medium without the addition of any nitrogen source as a control. The influence of initial pH was examined across a range of 5.5 to 8.5. The effect of biomass size was also examined across the range of 1 to 10%. The effect of initial substrate concentrations ranging from 100 mgL^{-1} to 1000 mgL^{-1} were used. Similarly, the influence of temperature was also examined across the range of 25°C to 50°C. The strain's capacity to utilize and breakdown anthracene and phenanthrene in BH media were evaluated using a UV-VIS spectrophotometer (Spectrum-Lab 7525) to measure the rise in turbidity of the BH broth at 24-hour intervals at OD 600 nm.

Quantification of anthracene and phenanthrene degradation

Following the incubation period (72 h), the residual amount of anthracene and phenanthrene in each triplicate flask was extracted with 20 mL of ethyl acetate. The extraction yielded two layers and the upper organic layer was removed and measured using a spectrophotometer by the optical density (OD) at respective λmax (anthracene 380 nm and phenanthrene 280 nm) and the formula below (1) (Rabani et al., 2020) was used to compute the percentage degradation:

$$\% \text{ Degradation Efficiency} = \left(\frac{C_0 - C_f}{C_0}\right) \times 100 \qquad (1)$$

C_0= Initial concentration in control
C_f= final concentration in test

Biodegradation experiment

Isolate grown on nutrient agar plates was inoculated and grown in LB broth for 24 hours before being extracted as pellets after 10 min of centrifugation at 4000 rpm. The pellets were standardized BH medium to make a concentrated bacterial solution (1.00 ± 0.02) with a spectrophotometer. Degradation of each PAH by the bacteria strain was assessed in autoclaved 250 mL Erlenmeyer flask. Anthracene and phenanthrene were dissolved in acetone at 600 mgL^{-1} and 400 mgL^{-1} concentrations, respectively. 1 mL of the solution was pipetted into a sterile empty flask, made up to 99 mL with BH liquid, and was left in a shaker overnight to get rid of the acetone solvent. Then, all flasks (anthracene and phenanthrene) except for control flasks received a 4 % (v/v) inoculum solution of the isolate, and they were incubated for 72 hours at 35 °C in an incubator shaker at 150 rpm. After the incubation period, the medium was passed through UV light to stop the microbial activities and to release the cytoplasmic content, ethyl acetate was used as a solvent to extract the residual PAH (anthracene and phenanthrene) and their degraded products residue from each medium. 20 mL of ethyl acetate was added to the medium to extract the

remaining PAH. The mixture was then sonicated for 10 minutes before being separated using a separating funnel. The medium was separated into two layers, with the upper layer containing the remaining PAH and its breakdown product. The top layer's PAH was collected. Allowing the extract to dry, the volume of each extract was increased to 100 mL by adding more ethyl acetate. The residue was stored in a refrigerator at 40°C before being transferred to GC vials for analysis. Control samples were those that had not been inoculated.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

Agilent Gas Chromatography (GC 7890B, MSD 5977A, Agilent Tech) with a DB 35- MS Capillary Standard Non-polar column (30 m×0.25 mm×0.25 μM) film thickness was used to measure PAH degradation. The organic phase was examined by GC-MS using 1 microliter of sample. The GC-MS analysis was performed using a gas chromatograph outfitted with a split-split less injector (split ratios of 50:1). The carrier gas had a steady flow of 1 mL/min and was helium. The oven was first set at 40°C for five minutes. The injection, transfer, and ionization source temperatures were all 270°C at 37.1 Kpa. Over the course of 6.5 to 85 minutes, the mass spectrometer was run in full scan mode in electron impact (EI) mode at 70 electron volts (EV) from 85 to 450 m/z. Temperatures for the injector and detector were 270° C and 280°C, respectively. To calculate the percentage degradation efficiency of anthracene and phenanthrene by strain BUK_BTEG1 from the chromatogram generated, equation (2) below was used (Mohan et al., 2019). On the other hand, to confirm and identify the metabolites of various PAHs (anthracene and phenanthrene) degradation, the NIST GC–MS library program search was used to relate the m/z fragmentation pattern of the detected metabolite to the ones from the reference Database (NIST, 2017).

$$\% \text{ Degradation efficiency} = = \left[\frac{\text{Standard AUC} - \text{Sample AUC}}{\text{Standard AUC}}\right] \times 100 \quad (2)$$

AUC = Area under the curve/peak area

3. Results and discussions

In the current study, Bushnell-Haas (BH) supplemented with either anthracene or phenanthrene as the only sole source of carbon and energy was used for isolation and screening for a potential PAH-degrading bacteria strain obtained from petrochemical-contaminated soil samples. The biodegradation potential of the strain was indicated by the formation of turbidity as an index of biomass growth in the BH enrichment medium.

For bacterial identification, it has been discovered that 16S rRNA gene sequencing maintains a respectable level of accuracy and dependability (Hong and Farrence, 2015). In order to identify the isolate, a small segment of the 16S rRNA gene was amplified using PCR. When the amplified product for the isolate was run on 1.5 percent agarose gel electrophoresis, it revealed around 1500 bp (**Fig. 1A**). Depending on the species, segment amplified, or kind of primers used, other studies have previously reported comparable ranges of 16S rRNA gene amplification products for the genus Pseudomonas between the ranges of 1200 and 1500 bp (Görtler and Stanisich, 1996; Widada et al., 2002; Godini et al., 2019). The gene amplicon was successfully sequenced and blasted. The isolate was identified as a member of the genus Pseudomonas according to blast results in the NCBI GenBank revealing that the isolate showed high similarities to the first forty representatives that were of genus

Pseudomonas, revealing the highest similarity of 99.6% to Pseudomonas stutzeri strain AKVG5, followed by Pseudomonas stutzeri strain FN9 98.8%.The sequence was deposited underthe accession number OM039162 into the NCBI GenBank database. The 16S rRNA gene sequence underwent phylogenetic analysis using the neighbor-joining tree technique (**Fig. 1B**).

The phylogeny and evolutionary analysis showed parent clads with Pseudomonas stutzeri strain VKMB-97 5and sub-clads of Pseudomonas stutzeristrain NERC 14165, Pseudomonas stutzeri strain DM51990, Pseudomonas stutzeristrain P2and BUK-BTEG1 with a bootstrap valueof 95% for the parent, indicating a closer relatedness of these organisms (**Fig. 1B**).

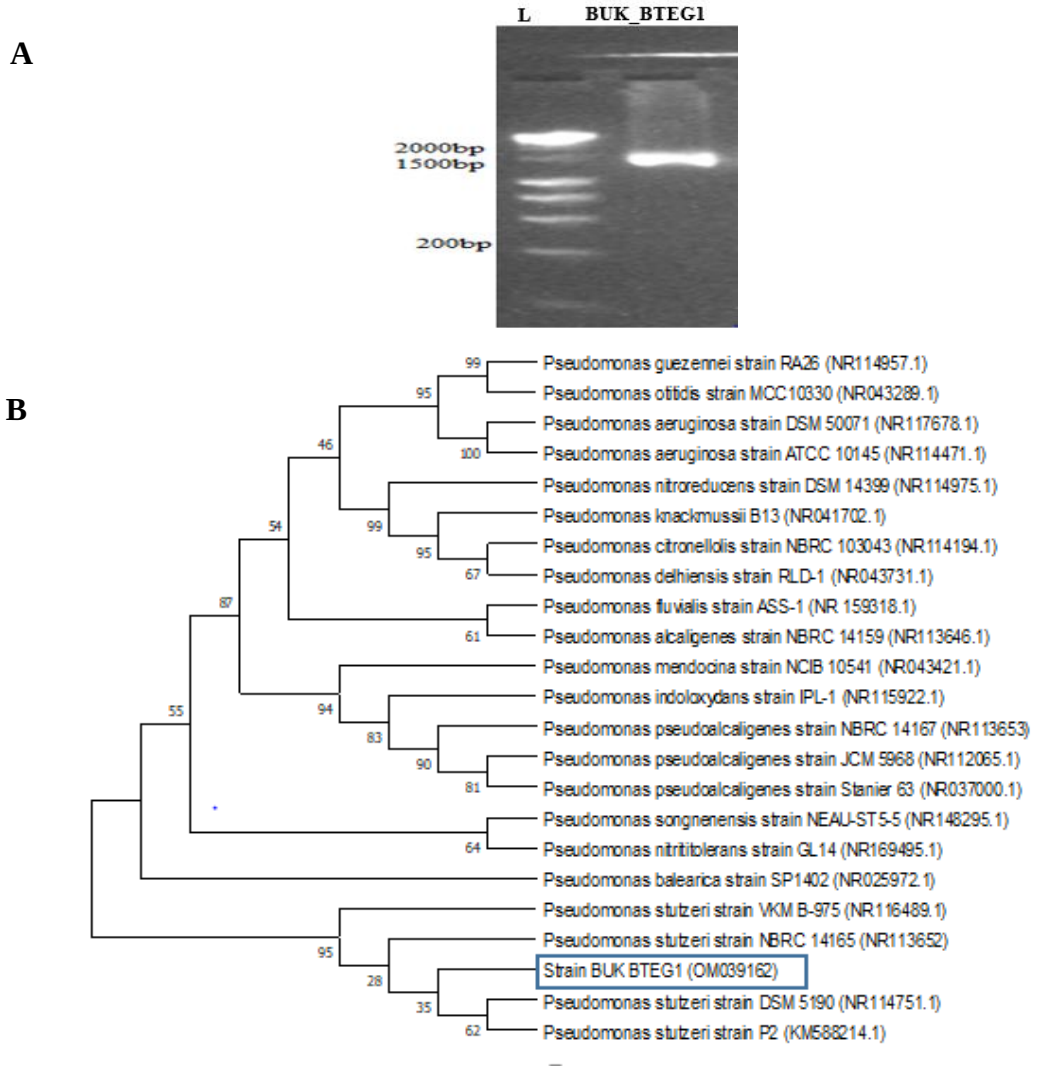

Fig. 1. Visualization of the 16S rRNA PCR amplification product on the BUK BTEG1 strain's Agarose gel electrophoresis. L stands for Bioline's Hyper Ladder-2K marking (Lot Number H4-q111B) (**A**); Phylogenetic and evolutionary relationships of taxa of strain BUK-BTEG1. The rectangle denoted the strain's evolutionary position. The Neighbor-Joining approach was used to infer the evolutionary history. The accession numbers are included beside the species names (**B**)

Therefore, strain BUK-BTEG1 was tentatively designated as *Pseudomonas stutzeri* strain BUK-BTEG1. Several studies for anthracene and phenanthrene-degrading bacteria belonging to the genera Pseudomonas were reported (Li et al., 2021; Singh and Tiwary, 2017; Nwinyiet al., 2016).

Bioremediation is often limited by environmental, physical, and chemical factors (Naik and Duraphe, 2012). The most important factors that can be detrimental to bacterial growth and ability to digest PAHs effectively and efficiently include nitrogen source, pH, substrate concentration, bacteria inoculum size, and temperature. Controlling these factors is important for biodegradation by microbial strain (Fareezet al., 2021).

The availability of suitable nitrogen sources, in addition to the carbon source, is one of the most crucial factors in the biodegradation processes (Ibrahim et al., 2020). Nitrogen-containing compounds are being integrated into biodegradation byproducts as well as enzymes and cofactors in the metabolic pathways involved in PAH biodegradation. Different nitrogen sources may hinder or stimulate bacterial growth and degradation.Any microorganism needs nitrogen for growth and metabolism, as well as for the production of RNA, DNA, and proteins. The strain BUK BTEG1 showed maximal growth and PAH consumption when ammonium sulphate, ammonium chloride, or sodium nitrate was utilized, with the highest performance seen when utilizing ammonium nitrate (out of the four organic and inorganic nitrogen sources evaluated in this study) (**Fig. 2A**)

The findings of this study on the impacts of various nitrogen sources are consistent with those of Merike et al. (2017) and Mujahid et al. (2015).The outcome, however, defies the claims made by Dudhagara et al. (2018), Sachaniya et al. (2010), and Al-Dossary et al. (2021) that sodium nitrate was the optimum nitrogen source for degradation.

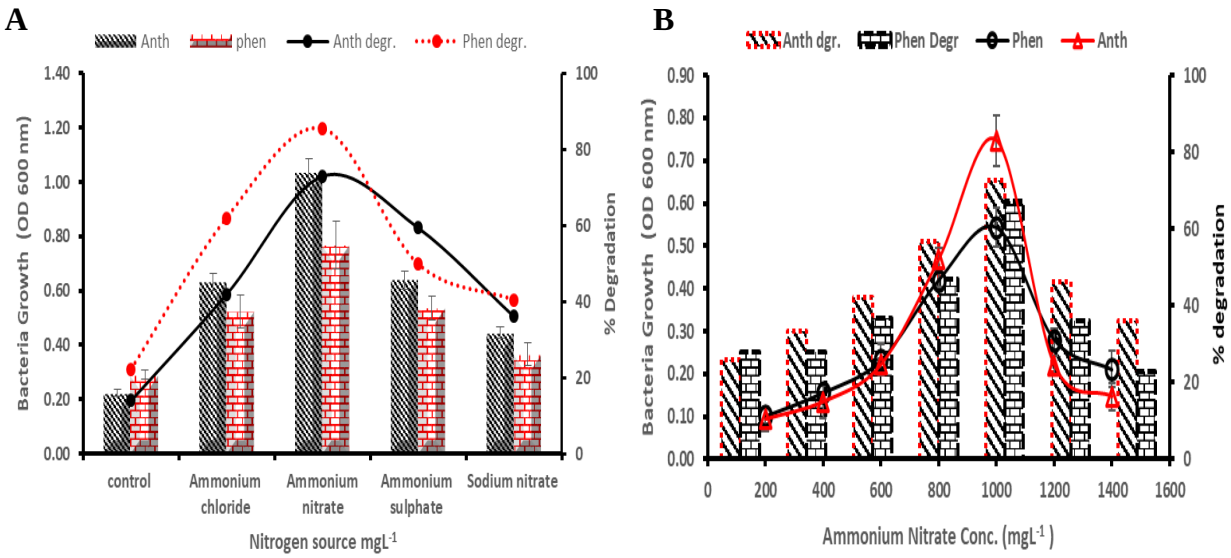

Fig. 2. The effect of nitrogen sources (**A**) and ammonium nitrate concentration (**B**) on bacterial growth and degradation of various PAH (anthracene and phenanthrene) by strain BUK_BTEG1 Data represent mean ± standard deviation of triplicate determination.

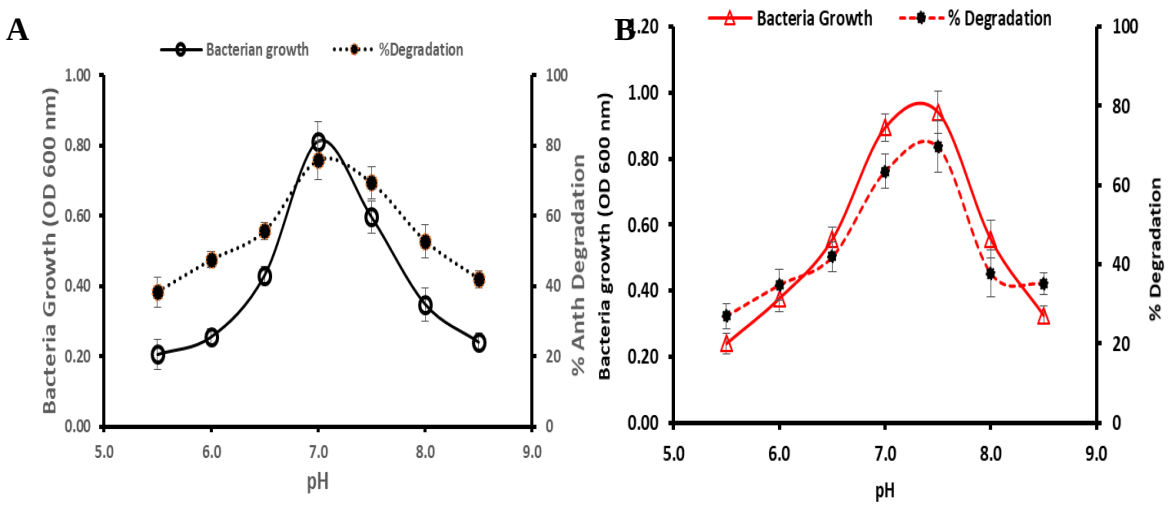

Fig. 3. The effect of initial pH on growth of strain BUK_BTEG1 and anthracene (**A**) and phenanthrene (**B**) degradation. Data represent the mean ± standard deviation of triplicate determinations.

It is critical to have access to a suitable nitrogen supply, but the concentration is equally necessary (Fareezet al., 2021). Investigation into the effects of various ammonium nitrate concentrations on the strain growth and the degradation of various PAHs revealed that 1000 mgL^{-1} was the ideal concentration (**Fig. 2B**). The rate of PAH breakdown slows down when the concentration is below the optimum. This might be because there isn't enough ammonium nitrate available to support the bacterium's rate of replication, or it might be because the media's increased pH is a result of the presence of ammonia. The atmosphere becomes more alkaline when there is too much ammonia, which slows down the degradation rate. Similarly, the results agree with Singh and Tiwary (2017), who reported optimum degradation with 1000 mgL^{-1} ammonium nitrate, but differ from Amani et al. (2020) and Fareez et al. (2021), who described 3000 mgL^{-1} ammonium nitrate as the optimum for degradation by bacteria consortium and Bacillus species.

During the microbial breakdown of PAHs as a solitary source of carbon and energy, the pH of the surrounding environment plays an important role in the process (Abatenhet al., 2017). Most microorganisms thrive at pH levels close to neutral. Microbial tolerance to pH stress is frequently aided by the ability of the microbes to physiologically alter their cell membrane to aid intracellular pH control. At varied initial pH values ranging from pH 5.5 to pH 8.5, the effects of pH on the bacterium growth and various PAH degradation were examined., and an optimum pH of 7.0 was identified for anthracene **(Fig. 3B**) while pH 7.5 was identified optimum for degradation of phenanthrene (**Fig. 3A)**. In the current investigation, microbial growth and PAH breakdown were both at their peak between pH 7 and 7.5, with performance rapidly declining at higher alkaline pH values. pH fluctuations in a shake flask setup are mostly caused by the buildup of metabolic wastes, which may be compensated for by using appropriate buffer systems. The inclusion of phosphorus-containing chemicals in the experimental buffer solution may have provided nutritional support to the microbial cells, promoting bacterial growth (Fareezet al., 2021). The ideal pH for bacteria consortiums or individual strains to break down hydrocarbons is between 6.5 to 7.5,

according to other studies, which is consistent with the present findings (Al-Dossaryet al., 2021; Bibi et al., 2018; Elufisanet al., 2020; Singh and Tiwary, 2017).

Biomass dosage also played a major role in the PAH degradation process, the amount of bacteria population in the medium affects the acclimatization of the cell and the enzyme levels synthesized to facilitate cell metabolism (Koutsoumanis and Sofos, 2005). It is essential to determine the volume of the inoculum to achieve maximum PAH degradation. Over a 1–10% (v/v) range, the effect of the initial biomass population was investigated. From the result presented in (**Fig**. **4A** and **B**). Bacterial growth and breakdown were optimum for both PAHs with a biomass dosage of 4%, after which there was a decline.

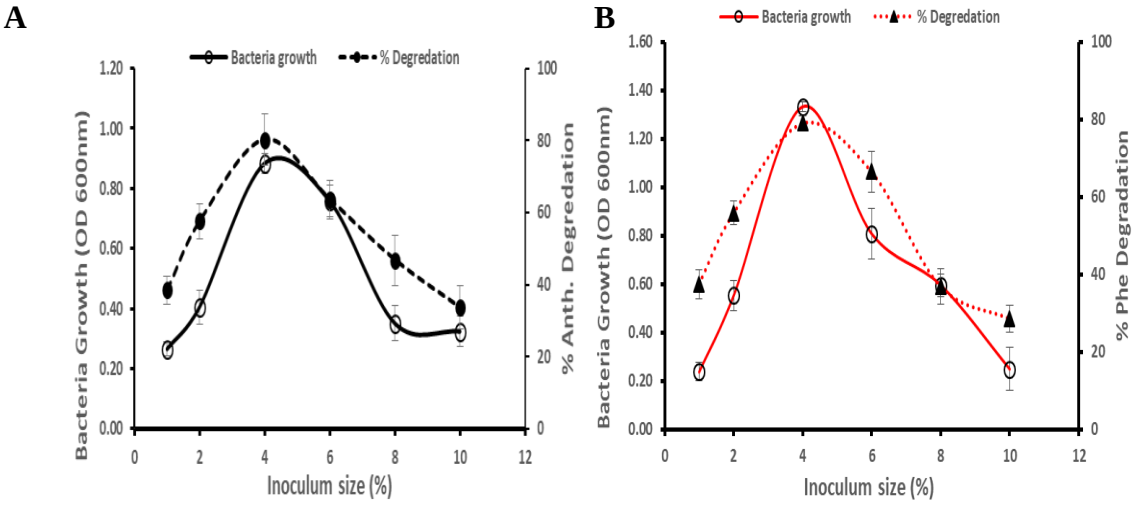

Fig. 4. The effect of inoculum size on bacterial growth and degradation of anthracene (**A**) and phenanthrene (**B**) by strain BUK_BTEG1. Data represent the mean ± standard deviation of triplicate determination.

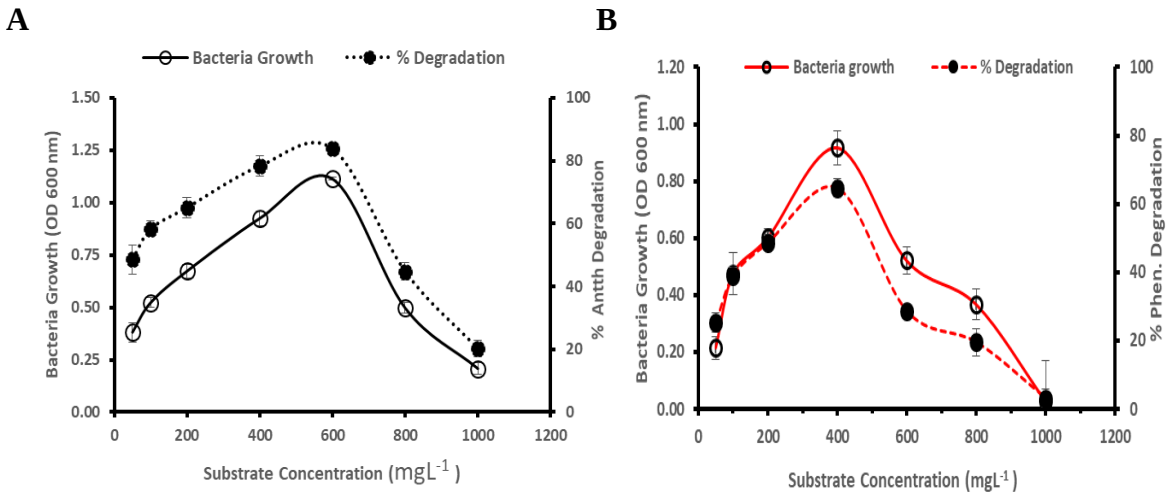

Fig. 5. The effect of anthracene (**A**) and phenanthrene (**B**) concentration on bacterial growth and degradation by strain BUK_BTEG1. Data represent mean ± standard deviation of triplicate determination

The decline in growth and degradation observed at higher inoculum beyond the optimum may be attributed to a rapid increase in cell density that competes for the limited nutrients, resulting in the death of less competent cells, or it may most likely result from nutritional depletion and a lack of total dissolved oxygen accessible to the cells (Ghosal et al., 2016).

It is known that some microbial species can withstand PAH concentrations that are high (Bibi et al., 2018). Substrate concentration plays an essential role in bacteria growth and PAH degradation. Any viable biodegradation bacterial strain should be able to tolerate and break down a PAHs high concentration. Anthracene and phenanthrene initial concentrations ranging from 100 mgL^{-1} to 1000mgL^{-1}were examined for their impact on the strain growth rate and degradation. Optimum growth and degradation by the strain were achieved at a concentration of 600 $mgL^{-1}$and 400 $mgL^{-1}$for anthracene **(Fig. 5A**) and phenanthrene **(Fig. 5B)** respectively following 72 h of incubation.

For each of the PAHs, the strain's growth and degradation decreased quickly at starting PAH concentrations above the optimal levels. The data obtained indicate that concentrations above the optimum may have been toxic to the strain, leading to a proportional decrease in growth and degradation efficiency. Similar results were reported by Patel et al., (2018) for high degradation at initial concentrations of 50 mgL^{-1}–1000 mgL^{-1}, which dramatically decreased at a concentration over 500 mgL^{-1}. Furthermore, a similar result is also reported by Bibi et al., (2018) whose optimum concentration was 1000 mgL^{-1}, also with an observed proportional decline in growth and degradation at a concentration greater than the optimum. This further suggests that a higher concentration of PAH may be toxic to the metabolic activity which in turn affects the growth and degradation efficiency of the bacteria. Several other findings also reported optimum degradation at low PAH concentration in contrast to the findings of this study (Singh and Tiwary, 2016; Praveen, 2019).

One of the most significant physical factors impacting the growth and proliferation of microorganisms is temperature. There is an ideal temperature for each enzyme-mediated breakdown process (Abatenh et al, 2017). The desolations of PAHs and bacteria's capacity to metabolize them may be impacted by an increase in temperature (Bhattacharya et al., 2011). For example, high temperatures can make PAHs more soluble and accessible while decreasing oxygen solubility, which can primarily impair the activity of aerobic bacteria (Bibi et al., 2018). As a result, earlier studies have focused on intermediate temperatures rather than high or low temperatures. Similar to the previous research, high anthracene and phenanthrene degradation were seen at 35 °C in the current investigation (**Fig. 6A** and **B**), which can be related to the optimal growth conditions for the strain. As the research organism was a mesophilic bacterium, additional investigations have discovered that the ideal temperatures for PAH breakdown were between 30 and 40°C. Bisht et al. (2015) and Nzila et al. (2018) similarly found that *Pseudomonas citronellolis* strain PHC3Z1A and a mixed culture of *Pseudomonadales*, Actinobacteria, *Caulobacterales*, *Rhizobiales*, and *Xanthomonadales* degraded best at 35°C (Ashok-Kumar et al., 2018).

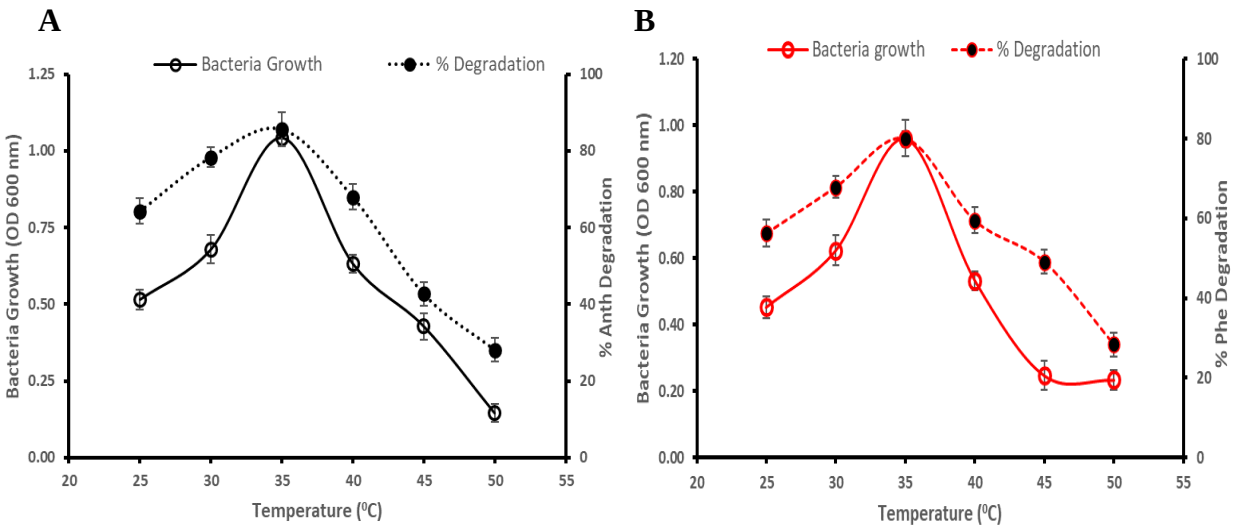

Fig. 6. The effect of temperature on the growth and degradation of anthracene (**A**) and phenanthrene (**B**) by strain BUK_BTEG1. Data represent the mean ± standard deviation of triplicate determination.

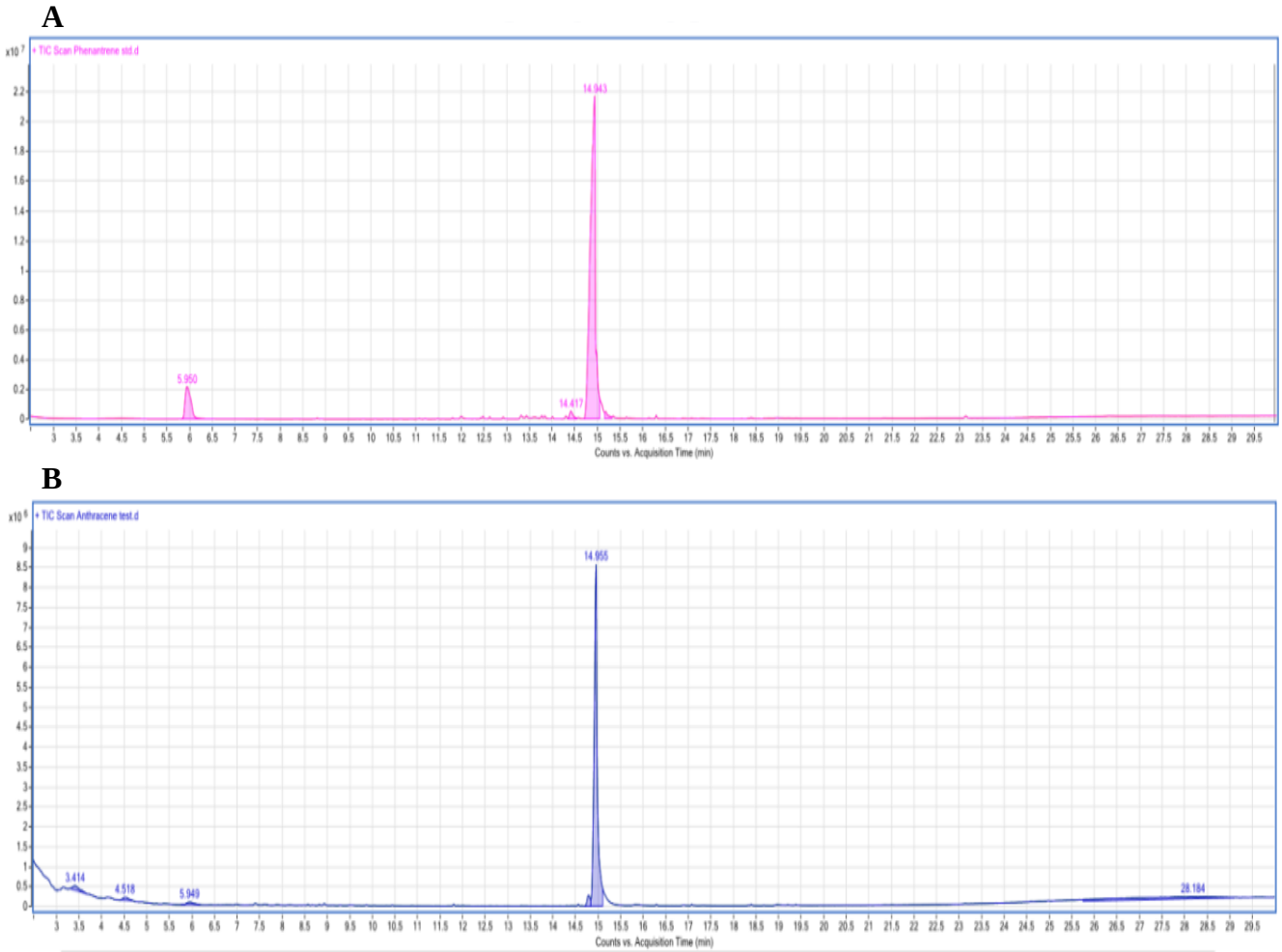

Fig.7. GC-MS chromatogram of anthracene (uncultured medium (**A**) and cultured medium (**B**))

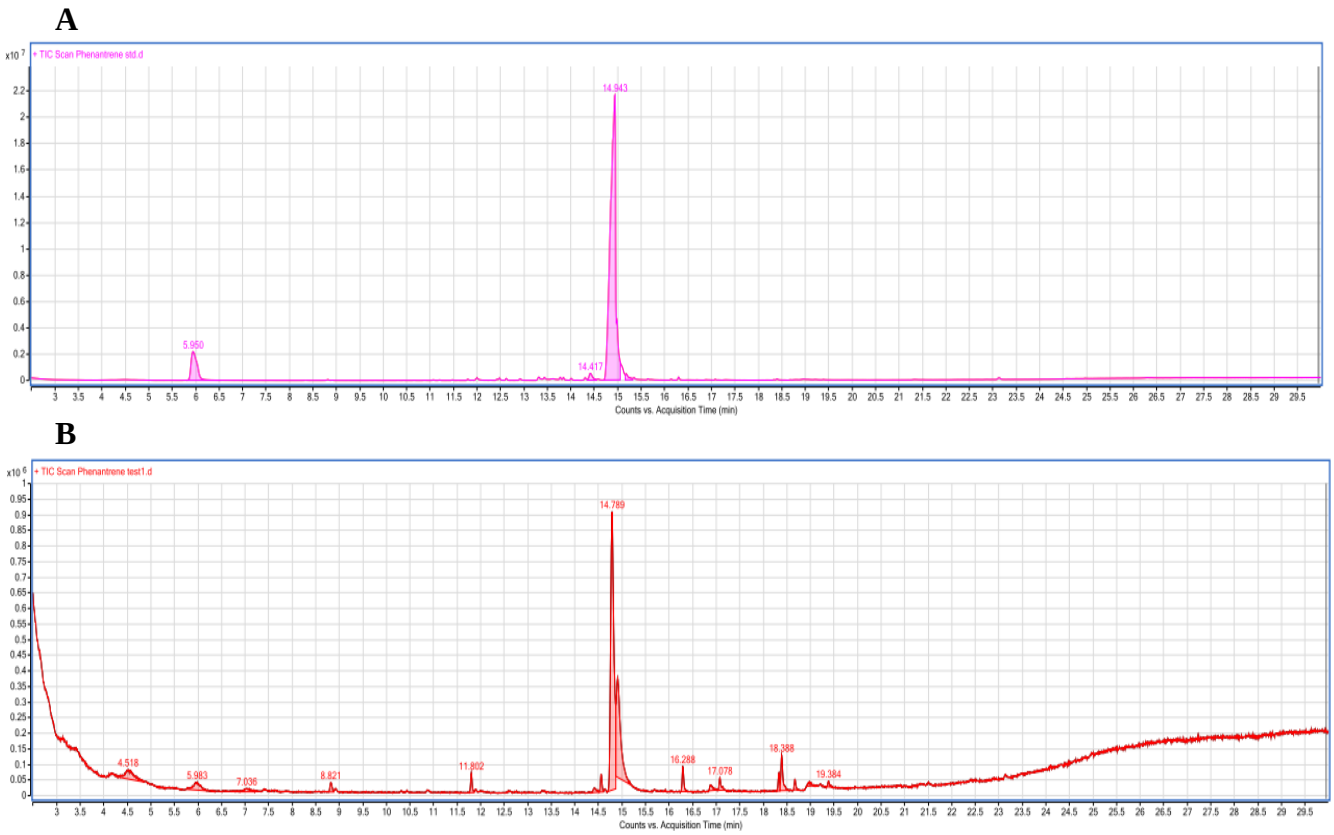

Fig. 8. GC-MS chromatogram of phenanthrene (uncultured medium (**A**) and cultured medium (**B**)) 400 mgL^{-1}at pH 7.0 ± 0.2 shaken at 120 rpm incubated at 35 ºC for 120 hours)

The biodegradation of PAHs may be significantly enhanced by optimizing environmental factors. The modification of environmental factors to promote the development and enzymatic activity of the existing bacteria is known as bio-stimulation (Bibi et al., 2018). The effects of 1000 mgL^{-1} NH_4NO_3, 35°C, and pH 7.0 on each of the PAH (600 mgL^{-1} anthracene, and 400 mgL^{-1} phenanthrene) degradation by strain BUK_BTEG1 were evaluated here using GC-MS to get the area under the curve (AUC) for both non-culture (**Fig. 7A** and **8A**) and cultured (**Fig. 7B** and **8B**) samples of the anthracene and phenanthrene respectively. Under bio-stimulation, the total percentage of anthracene and phenanthrene degradation was 72.09%, and 98.81% respectively following 72 h incubation. A substantial literature has reported high PAH degradation within that range and that corresponds to those shown by this strain (Singh and Tiwary, 2017; Salamat et al., 2018; Shehu et al., 2021).

The use of GC-MS methods is crucial for highlighting certain structural characteristics that might indicate intermediary metabolites in the biodegradation of anthracene and phenanthrene. The primary metabolites of the anthracene and phenanthrene biodegradation pathway were found in the current investigation to be derivatives of salicylic acid, catechol, benzyl-pyruvic acid, and phthalic acid (**Table 1**). These findings suggested that the PAHs degradation route by strain BUK BTEG1 followed a similar course to that described for *Pseudomonas* sp. strain Jpyr-1 and strain CECT 930 (Moscoso et al., 2015) *Pseudomonas otitidis* strain P4 (Singh and Tiwary, 2016) and *Pseudomonas stutzeri* P2 (Singh and Tiwary, 2017).

Table 1.GC-MS data for the metabolites of anthracene and phenanthrene obtained from the organic extracts of the cultures and resting cell incubations ofstrainBUK_BTEG1

Substrate	Retention Time	m/z	Suggested metabolites	Suggested structure
Anthracene	8.542	210.28	1,2-Dihydroxy anthracene	$C_{14}H_{10}O_2$
	5.949	122.12	Salicylaldehyde	$C_7H_6O_2$
	4.844	110.11	Catechol	$C_6H_6O_2$
	28.18	196.21	6,7-Benzocoumarin	$C_{13}H_8O_2$
Phenanthrene	14.532	262.41	3,4-Dihydroxyphenanthrene	$C_{14}H_{10}O_2$
	16.849	334.43	Phthalic acid derivatives	$C_{15}H_{13}NO_3$
	18.926	188.25	1-Hydroxy-2-naphthoic acid	$C_{11}H_8O_3$
	19.052	160.17	1,2-Naphthalene diol	$C_{10}H_8O_2$
	4.844	110.12	Catechol	$C_6H_6O_2$

Conclusions

Interaction *Pseudomonas* strain BUK BTEG1 was isolated from petrochemical-contaminated soil. The isolate effectively uses and degrades anthracene and phenanthrene as its only source of carbon and energy. The optimum degradation rate of anthracene and phenanthrene were achieved at ammonium nitrate, substrate concentration of 600 mgL^{-1} and 400 mgL^{-1} respectively, 35°C temperature and pH of 7.0 for anthracene while 7.5 for phenanthrene, inoculum size of 4% (v/v) and 72 hours of incubation time. Under optimal conditions, the strain could degrade around 72% and 99% of anthracene and phenanthrene at concentrations of 600 mgL^{-1} and 400 mgL^{-1}, respectively, in 72 hours. The presence of major metabolites of the pathway, namely catechol, salicylic acid, and phthalic acid derivatives, was revealed by gas chromatography-mass spectrometry analysis of anthracene and phenanthrene degradation products, indicating the representative of *Pseudomonas* as the significant PAHs degrader. As a result, the strain has excellent potential for bioremediation of polycyclic aromatic hydrocarbon polluted settings.

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. Abatenh, E, Gizaw, B, Tsegaye, Z, and Wassie, M (2017) Review Article Application of microorganisms in bioremediation-Review. Journal of Environmental Microbiology. 1(1):2–9.
2. Al-Dossary MA, Abood SA, and First HTA (2021) Effects of physicochemical factors on PAH degradation by *Planomicrobium alkanoclasticum*. Remediation Journal. 31(2):29–37.
3. Amani N, Mohd F, Tao W, and Hamzah N (2020) Establishing the Order of Importance Factor Based on Optimization of Conditions in PAHs Biodegradation Establishing the Order of Importance Factor Based on Optimization of Conditions in PAHs Biodegradation. Polycyclic Aromatic Compounds. 0(0): 1–15. https://doi.org/10.1080/10406638.2020.1833049
4. Bibi N, Hamayun M, Khan SA, Iqbal A, Islam B, Shah F, Khan MA, and Lee I

(2018) Anthracene biodegradation capacity of newly isolated rhizospheric bacteria *Bacillus cereus* S13. PLoS ONE. 13(8): e0201620. https://doi.org/10.1371/journal. pone.0201620

5. Bisht S, Pandey P, Bhargava B, and Sharma S (2015). Bioremediation of polyaromatic hydrocarbons (PAHs) using rhizosphere technology. Brazilian Journal of Microbiology. 46**(**1):7-21. https://doi.org/10.1590/S1517-838246120131354
6. Chowdhury R, Kartik D, Ahamed F, Shawkat A, and Mohammad U (2017) Biodegradation of anthracene and phenanthrene by bacteria isolated from oil-contaminated soil of Bangladesh, Chemistry and Ecology. doi: 10.1080/02757540.2017.1382481
7. Dudhagara DR, Dave BP, Dudhagara R, and Dave BP. (2018) Mycobacterium as Polycyclic Aromatic Hydrocarbons (PAHs) Degrader.J. Sci and Healthcare Res. https://doi.org/10.5772/intechopen.73546
8. Elufisan TO, Rodríguez-luna IC, Oyedara OO, Sánchezvarela A, Hernández-mendozaA, Dantán E, Paz-gonzález AD et al. (2020) The Polycyclic Aromatic Hydrocarbon (PAH) degradation activities and genome analysis of a novel strain *Stenotrophomonas sp*. *Pemsol* isolated from Mexico. https://doi.org/10.7717/peerj.8102
9. Fareez A, Roslee A, Gomez-fuentes C, Zakaria NN, Shaharuddin NA, Zulkharnain A, Khalil KA, et al. (2021) Growth Optimisation and Kinetic Profiling of Diesel Isolated from Trinity Peninsula, Antarctica. Biology. 10, 493. https://doi.org/ 10.3390/biology10060493
10. Fritt-rasmussen J, Wegeberg S, and Gustavson K (2015) Review on Burn Residues from In Situ Burning of Oil Spills in Relation to Arctic Waters. Water Air Soil Pollution: 226:329. https://doi.org/10.1007/s11270-015-2593-1
11. Ghosal D, Ghosh S, Dutta TK, and Ahn Y (2016) Current State of Knowledge in Microbial Degradation of Polycyclic Aromatic Hydrocarbons (PAHs): A Review. Frontiers in Microbiology. **7**:1369. https://doi.org/10.3389/fmicb.2016.01369
12. Godini K., Mohamad RS, Hamed T, Omid Zarei, Karimitabar Z, Yarahmadi Z. & Rez MA (2019) Biochemical and molecular characterization of novel PAH-degrading bacteria isolated from polluted soil and sludge. Petroleum Science and Technology. 37:15:1763-1769, DOI: 0.1080/10916466.2019.1575864
13. Gupte A, Tripathi A, Patel H, Rudakiya D, and Gupte S (2016) Bioremediation of Polycyclic Aromatic Hydrocarbon (PAHs): A Perspective. The Open Biotechnology Journal`. 10(1): 363–378. https://doi.org/10.2174/1874070701610010363
14. Haritash A. K and Kaushik C, P (2009) Biodegradation Aspects of Polycyclic Aromatic Hydrocarbons (PAHs): A Review.” Journal of Hazardous Materials. 169:1–15. https://doi.org/10.1016/j.jhazmat.2009.03.137
15. Hong S, Farrence CE. (2015) Is it essential to sequence the entire 16S RRNA gene for bacterial identification? American Pharm Rev. 18 (7):1–7.
16. Koutsoumanis KP, and Sofos JN, (2015) Effect of inoculum size on the combined temperature, pH and a w limits for growth of *Listeria monocytogenes*. Int J Food Microbiol. 104:83–91. https://doi.org/10.1016/j.ijfoodmicro.2015.01.010 PMid: 16005535
17. Li W, Liu Y, Li D, Wang L, Mao Z, and Wang Q. (2021) Enhanced Biodegradation of Anthracene by Multi-Substrate Progressive Domestication of a Mixed

Microbial Consortium with *Pseudomonas_AeruginosaDM3*. 1–18. http://dx.doi.org/10.21203/rs.3.rs-292903/v1

18. Merike J, Viggor S, Heinaru E, Naanuri, E, Mehike M, Leito I, and Heinaru A (2017) Strategy of *Pseudomonas pseudoalcaligenes* C70 for effective degradation of phenol and salicylate. PLoS ONE: 1–17. https://doi.org/10.1371/journal.pone.0173180
19. Mohan LK, Kumari V, and RoyS. (2019) *Phenylobacterium Korensee* Best Indigenous Petroleum Hydrocarbon Degrading Bacteria Isolated from Contaminated Soil of Bahror, Alwar Region, India. International Journal of Contemporary Research and Review. 10(08), 20203–20211.
20. Moscoso, F., Deive, F.J., Longo, M.A. et al. Insights into polyaromatic hydrocarbon biodegradation by *Pseudomonas stutzeri* CECT 930: operation at bioreactor scale and metabolic pathways. Int. J. Environ. Sci. Technol. 12:1243–1252 (2015). https://doi.org/10.1007/s13762-014-0498-y
21. Mujahid TY, Wahab A, Padhiar SH, Subhan SA, Baloch MN, and Pirzada ZA (2015) Isolation and Characterization of Hydrocarbon Degrading Bacteria from Petrol Contaminated Soil. Journal of Basic and Applied Sciences. 11:223-231.
22. Naik MMG, and Duraphe MMD (2012) Review paper on parameters affecting bioremediation. International Journal of Life Science and Pharma Research. 2(3): L77–L80.
23. Neeraja P, Soma Shanker MNC, Ravikumar M. (2013) Isolation and characterization of anthracene degrading bacteria from soil contaminated with petroleum wastes. Int J Res Pharm Sci. 4(3):380–385.
24. NIST: National Institute of Standard and Technology (2017) Mass spectra Library (NIST17) and NIST mass spectra search program standard reference data program. V2.3 for windows
25. Nwinyi OC, Ajayi OO, and Amund OO (2016) Degradation of polynuclear aromatic hydrocarbons by two strains of *Pseudomonas*. Brazilian Journal of Microbiology. 47(3):551–562. https://doi.org/10.1016/j.bjm.2016.04.026
26. Nzila, A., Sankara, S., Al-momani, M., and Musa, M. M. (2018) Isolation and characterization of bacteria degrading polycyclic aromatic hydrocarbons : phenanthrene and anthracene. Archives of Environmental Protection. 44(2):43–54. https://doi.org/10.24425/119693
27. Oaikhena EE, Makaije DB, Denwe SD, Namadi MM, and Haroun AA. (2019) Bioremediation Potentials of Heavy Metal Tolerant Bacteria Isolated from Petroleum Refinery Effluent. American Journal of Environmental Protection. 5**(**2):29-34. doi: 10.11648/j.ajep.20160502.12y, 28–34.
28. Patel AB, Krutika M, Jain K, and Madamwar D (2018) Development of mixed bacterial cultures DAK11 capable for degrading mixture of polycyclic aromatic hydrocarbons (PAHs). Bioresource Technology. 253:288-296
29. Praveen R. (2019) Isolation, Characterization, and Identification of Anthracene Degrading Bacteria Occurring in Oil Contaminated Soils of Mechanical Workshops. International Journal of Science and Healthcare Research. 4(2):4–6.
30. Rabani MS, Sharma R, Singh R Gupta MK. (2020) Characterization and Identification of Naphthalene Degrading Bacteria Isolated from Petroleum Contaminated Sites and Their Possible Use in Bioremediation. Polycyclic Aromatic Compounds. 42(3):978-989. https://doi.org/10.1080/10406638.2020.1759663

31. Rabiu I. and Gimba, YM (2021) The use of Microorganisms in the Bioremediation of Xenobiotics in the Environment" Book Title: "Biotechnology for Sustainable Development" by Immortal Publications, India. In collections with the Dept. of Biosciences and Biotechnology Krishna University India. ISBN: 978-93-5437-925-3. https://www.immortalpublications.com
32. Sachaniya BK, Gosai HB, Panseriya HZ, and Dave BP. (2020) Chemometrics and Intelligent Laboratory Systems Bioengineering for multiple PAHs degradation for contaminated sediments: Response surface methodology (RSM) and artificial neural network (ANN). Chemometrics andIntelligent Laboratory Systems. 202(4):104033. https://doi.org/10.1016/j.chemolab.2020.104033
33. Salamat N, Lamoochi R, and Shahaliyan F. (2018) Metabolism and removal of anthracene and lead by a *B .subtilis* - produced biosurfactant. Toxicology Reports. 5(11):1120–1123. https://doi.org/10.1016/j.toxrep.2018.11.004
34. Shehu U, Ahmad FA, Yusuf F, and Muhammad, F. (2021) Isolation and Identification of Anthracene Utilizing *Proteus vulgaris* from Oil Spill Contaminated Soil at NNPC Depot Kano State Nigeria. 24(10):46–53. https://doi.org/10.9734/JABB/2021/v24i1030246
35. Singh P and Tiwary BN (2016) Isolation and characterization of glycolipid biosurfactant produced by a *Pseudomonas otitidis strain* isolated from Chirimiri coal mines. India Bioresour. Bioprocess. 3:42.
36. Singh P, and Tiwary BN (2017) Optimization of conditions for polycyclic aromatic hydrocarbons (PAHs) degradation by *Pseudomonas stutzeri P2* isolated from Chirimiri coal mines. Biocatalysis and Agricultural Biotechnology. 10(1): 20–29. https://doi.org/10.1016/j.bcab.2017.02.001
37. Suzuki T, and Takizawa N (2019) Purification and enzymatic characterization of aldolase from *Rhodococcusopacus* and enzymatic formation of α, β -unsaturated ketones. Bioscience, Biotechnology, and Biochemistry. 00(00): 1–5. https://doi.org/10.1080/09168451.2019.1625262
38. Tamura K, Stecher G, and Kumar S (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution https://doi.org/10.1093/molbev/msab120.
39. White AJ, Bradshaw PT, Herring AH, Teitelbaum SL, Beyea J, Stellman SD, Steck SE, Mordukhovich, I. et al. (2016) Exposure to multiple sources of polycyclic aromatic hydrocarbons and breast cancer incidence. Environ Int.ahttps://doi.org/10.1016/j.envint.2016.02.009
40. Widada J, Nojiri H, Kasuga K, Yoshida T, Habe H, Omori T. (2002) Molecular detection and diversity of polycyclic aromatic hydrocarbon-degrading bacteria isolated from geographically diverse sites. Appl MicrobiolBiotechnol. 58(2):202-209. doi:10.1007/s00253-001-0880-9
41. Ya'u M, Bennett CN and Lawrence USE (2020) *Staphylococcus* sp. Strain my 83295f: a potential *p,p'*-ddt-degrading bacterium isolated from pesticide-contaminated soil. Acta Biologica Marisiensis Journal. 3(2): 22-35
42. Zelinkova Z, and Wenzl T (2015) The Occurrence of 16 EPA PAHs in Food – A Review. Polycyclic Aromatic Compounds. 35:248–284. https://doi.org/10.1080/10406638.2014.918550

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ISOLATION AND CHARACTERIZATION OF *BACILLUS* SPP. FOR PLANT GROWTH PROMOTING PROPERTIES

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Abstract: A group of free-living bacteria known as plant growth-promoting rhizobacteria (PGPR) inhabit the rhizosphere and aid root development. These rhizobacteria bacteria are vital to the growth of plants and can serve as bio-fertilizer and can enhance food security through green agricultural practices. They exhibit special features which make them potential candidates as bio-fertilizer. Isolation and characterization of rhizobacteria is the first step toward their utilization as bio-fertilizers. Ten rhizobacteria from two different rice farms were isolated and characterized for plant growth promoting properties. The isolated rhizobacteria were identified morphologically, microscopically, biochemically, and molecularly. Plant's growth promoting properties of these rhizobacteria was also analyzed which includes; Indole 3-acetic acid production (IAA), phosphate solubilisation, hydrogen cyanide production (HCN), ammonia production (NH_3), and zinc solubilisation. Out of the ten isolates, three were found to have the best plant growth enhancing properties and were therefore the best candidates as bio-fertilizers. 16SrRNA study and phylogenetic analysis was performed in order to unravel the specie of these three isolates and they were identified as *Bacillus subtilis*, *Bacillus niacini*, and *Bacillus cereus* with accession numbers OM184294, OM1842295 and OM184296 respectively. These isolates have the potential to be used as bio-fertilizer, which would significantly contribute to food security.

Keywords: *Bacillus*, rhizobacteria, plant growth, Rice farm, bio-fertilizer, food security

1. Introduction

The quest to preserve ecological integrity of the soil through the use of green agricultural revolution is gaining momentum. Indiscriminate use of fertilizer, pesticides and other chemicals to enhance plant's growth occasioned by population explosion has negative consequences that affect the soil quality and microbial community surrounding the soil (Gouda et al., 2018; Molnár et al., 2020). Even in ideal situations, plants can only use 50% of the chemical fertilizer applied to them while the remaining is leached through the soil and interfere with underground and surface waters (Sharma and Singhvi, 2017). The effects of fertilizer application to the soil may not be immediately visible due to the

buffering capability of the soil; however, with time, soil degradation and disturbance of mineral elements balance eventually emerges (Sharma and Singhvi, 2017). These practices are associated with pollution of the environment, increase in soil acidity which eventually alter the pH of the soil and disruption of the ecological balance of the microbial flora in the soil. Several researches have shown the negative effects of chemical pesticides application in order to control weeds and pests (Rani et al., 2021; Sabarwal et al., 2018). This prompted a search for sustainable and less harmful green agricultural practices that can enhance plant growth while preserving the ecological integrity of the soil (Adnan et al., 2020).

Over the years, microorganisms have been linked to plant nutrient supply for agricultural development and production (Aeron et al., 2020; Péterfi and Domokos, 2018). Furthermore, the use of microorganisms to enhance plant's growth has the potential to reduce the level of pollution in the environment, raise the yields of the crops, and reduce the negative consequences of developing antibiotic resistance by pests and making the environment safer for incoming generations. Applications of plant growth-promoting rhizobacteria (PGPR) are being promoted worldwide for the expansion of sustainable agriculture. Plant growth-promoting rhizobacteria are microbes that promote beneficial effects on plant development through direct or indirect mechanisms (Rai et al., 2020). These rhizobacteria's direct mechanisms include nutrient acquisition, phytohormone synthesis, siderophore generation, and antioxidant property enhancement (Nazir et al., 2018). Through the activation of the plant immune system against phytopathogens, PGPR can indirectly accelerate plant growth (Mustafa et al., 2019). Additionally, PGPR plays crucial functions in plant physiology, which include enhancing abiotic stress responses (Rai et al., 2020).

In addition to colonizing the rhizosphere of plants, PGPR can also grow in, on, or around plant tissues and stimulate plant growth through a variety of direct (such as phosphorus solubilization, nitrogen fixation, phytohormone production, etc.) or indirect (such as reducing pathogenic infection and/or mitigating abiotic stresses) mechanisms (Prasad et al., 2019). These PGPR are the most well-known beneficial microorganisms associated with plants and the best-performing bio-inoculants, having demonstrated excellent performance under controlled settings such as phosphate solubilization, phytohormone synthesis, siderophores generation, and nitrogen fixation (Basu et al., 2021).

Among the many species of bacteria that promote plant's growth, *Bacillus* spp. are the most widely distributed in the soil with several advantages over other species (Sansinenea, 2019). They shared several properties such as phosphate solubilisation, enhanced nitrogen acquisition, and siderophore production with other PGPR species. Furthermore, *Bacillus* spp. have the advantage of forming an endospore which can enable them survive high temperatures in the soil (Kaloterakis et al., 2021). They can withstand wide temperature variations, and also exhibit both the direct and indirect mechanisms of enhancing plant's growth. These and other properties make them suitable candidates for green-agricultural revolution for enhanced crop production (Saxena et al., 2020). Furthermore, the use of indigenous soil bacteria with potential plant's growth promoting properties that has already been adapted to the environment is being encouraged for easier utilization of such bacteria (Saxena et al., 2020). In this study, we aim to isolate and characterize a potential plant growth promoting *Bacillus* spp. from two

different sites; a rice farm in Kura local government area of Kano state, Nigeria, and Centre for dry land Agriculture, Bayero University, Kano, Nigeria.

2. Materials and methods

Study site

The study was conducted in the Plant Biology Department's botanical garden at Bayero University, Kano, Nigeria at coordinates 11°58′50″N, 8°28′46″E.

Collection of soil sample

Soil sample were collected from two sites; the first soil sample (Sample A) was gotten from Kura local government area, Kano State Nigeria (this soil is a rice cultivated soil). The second sample was collected from Centre for Dry Land Agriculture (CDA), Bayero University, Kano (The soil is used for cultivating rice). It was collected using hand trowel to dig up the soil to about 5cm depth; the temperature of the soil was recorded as 37˚C. 50g of the soil was collected in a zip lock transparent bag and transported to the lab for further analysis. To break up soil lumps before usage, samples were carefully mixed and run through a 0.4mm mesh sieve.

Bacteria isolation

The soil samples collected from both sites were brought to the microbiology laboratory for the isolation of the bacteria. The test tubes were arranged in a test tube rack; 9 ml of distilled water was measured and distributed in 10 different test tubes for each of the sample. Serial dilution was carried out on the soil samples. Nutrient agar was prepared according to the manufacturer's instruction and the isolates were sub-cultured into the prepared petri plates. They were then incubated at 37°C for 24 hours. Plate culture with discrete colony was used and the morphological characteristics of the colony were noted down (Garrity et al., 2005).

Microscopic examination of isolates

Glass slides were all labeled, small drop of normal saline was added on the slide. Bacteria isolates were picked with a wire loop and mixed with normal saline on the slide. After allowing it to air dry, the slide was heat fixed. After 60 seconds, a few drops of crystal violet were applied to the smear. After rinsing the slide with water, a drop of lugol's iodine was applied to the smear and left to sit for 60 seconds. After another washing with water, 95 percent ethanol was applied for 5 seconds. The slide was washed, blotted dry, and prepared for microscopic inspection (Garrity et al., 2005).

Morphological and biochemical identification

When compared to Bergey's manual, pure colonies of PGPR were identified and classified based on the findings of their Gram colouration tests, morphological traits, and biochemical characteristics (Garrity et al., 2005).

Screening of isolates for plant growth promoting properties

Phosphate solubilizing property

On a Pikovskaya's agar medium, bacterial isolates were tested for phosphate solubilization (PAM). Under usual conditions, bacterial culture was injected on PAM in the plate's center and maintained there for 5 days at 30-35 °C. Clear zones that formed surrounding the colonies demonstrated phosphate solubilizing properties. Results were displayed

as the zone's diameter in millimetres (Rai et al., 2020).

Production of indole acetic acid (IAA)

With a few minor adjustments, the technique of Mohite (2013) was used to quantify indole acetic acid (IAA). Test tubes were filled with 50 ml of nutritional broth that contained 0.1 percent DL tryptophan before being sterilized in an autoclave for 15 minutes at 121 °C. After allowing it to cool, the isolates were added to the prepared broth and maintained there for 72 hours at 28 °C.The culture was centrifuged for 10 minutes at 4°C (10,000 rpm). Orthophosphoric acid was added in two drops to the supernatant (2 ml), then, Salkowski reagent (50 ml of 35 percent perchloric acid and 1 ml of 0.5 ml $FeCl_3$ solution) was added to 4 ml of the supernatant. IAA manufacturing is indicated by the color pink. The optical density at 530 nm was captured using the spectrophotometer. IAA concentration was measured in µg/ml.

Production of NH_3

To see how bacterial isolates produced ammonia, peptone water was employed. The freshly produced culture was then added to the 10 ml of peptone water and maintained at 30 °C for 48–72 hours. 0.5 ml of Nessler's reagent was added to the mixture. Ammonia production was detected by the color changing from pale yellow to dark brown (Mohite, 2013).

Production of HCN

Glycine was supplemented with bacterial culture and streaked over nutrient agar medium. A Whatman filter paper that had previously been soaked in a particular solution (0.5 percent picric acid and 2 percent sodium carbonate (w/v) was used to cover the agar. Parafilm paper was used to seal the plates, which were then incubated at 36°C for four days. The creation of hydrogen cyanide is indicated by the development of orange or red color (Sehrawat et al., 2022).

Zinc solubilisation

Based on hollow formation on a solid basal medium containing 0.1 percent ZnO, isolated bacteria were tested for their capacity to dissolve zinc. The width of the resulting zone was measured and recorded (Hashemnejad et al., 2021).

Molecular identification of bacterial isolates

Genomic DNA isolation

Genomic DNA was isolated using Prepease genomic DNA isolation kit according to the manufacturer's instruction. Bacterial pellets from a pure bacterial culture cultured on nutrient broth were centrifuged for two minutes at 5000 rpm. The pellets were then homogenized in 100 µL of buffer, which contained 1.6 ml of 5 M sodium chloride, 5.48 g of sucrose, 1.57 g of tris, 10.16 ml of 0.5 M EDTA, and 2.5 ml of 20 percent SDS. After 30 minutes at 65 °C, 14 µL of 8 M potassium acetate (to a final concentration of 1 M), which was added, was incubated the particle was washed in 100 µL of ice-cold, 70% ethanol after the supernatant had been placed into a fresh 1.5 ml Eppendorf and 200 L of 100% ethanol had been added and vortexed. The pellet was then washed in 100 µL of ice-cold 70 percent ethanol, dried, and suspended in 100 µL of dH_20, which was then incubated at 65 °C for 10 minutes. The total concentration of DNA was then determined using nanodrop spectrophotometer.

Polymerase Chain Reaction (PCR)

KAPA Taq DNA polymerase was used to conduct the PCR procedure. The total volume of the reaction was 25 µL. The reaction mixture consists of 2 µL of each genomic DNA, 2.5 uL of 10 µM TaqA Buffer, 0.4 mM (0.85 µL) each

of the forward and reverse primers; Bact1442-F (AGAGTTGATCCTGGCTCAG) and Bact1492-R (GGTTACCTTGTTACGACTT), 1.25 mM (1.5 µL) of $MgCl_2$, 0.25 mM (0.2 µL) of dNTP The following conditions were used for the amplification: 5 min initial denaturation at 95 °C, followed by 35 cycles of 30 s at 94 °C (denaturation), 30 s at 60 °C (primer annealing), and 1 min at 72 °C for each cycle (extension). This was followed by a final extension of 10 minutes at 72^0C.

Gel electrophoresis

The 1.5 percent agarose gel used to separate the PCR products was stained with ethidium bromide. The size of the DNA fragment was measured using Ingenius' Syngene Gel Documentation System and compared to the Hyper Ladder-2K marker on a 1 percent agarose gel (CSL-AG500, Cleaver Scientific Ltd.) (IG31459). A positive result was the existence of a product with the anticipated size. The PCR product was then send for sequencing. DNA sequencing was performed according to manufacturer's instructions using the ABI Prism Big DyeTM 3730/3730XL Terminator Cycle Sequencing Ready Reaction Kit.

Alignment and phylogenetic analysis

Using BLAST at the National Center for Biotechnology Information, the 16S rRNA gene sequences of the isolated organisms were aligned and compared with the known 16S rRNA gene sequences in the Genbank database to find the closest database sequences. Using Clustal Omega for multiple sequence alignment, the isolate's 16S rRNA gene sequences were matched with sequences from the GenBank databases. The Neighbor-Joining method was used to infer the evolutionary record Molecular Evolutionary Genetics Analysis (MEGA 11.0) for Windows was used to create the phylogenetic tree. Finally, sequences were submitted to GenBank and were given an accession number.

3. Results and discussions

Plant growth-promoting rhizobacteria (PGPR) are rhizosphere bacteria that can promote plant development in response to various biotic and abiotic stressors (Aeron et al., 2020). In addition to *Rhizobium* and *Pseudomonas, Bacillus* are among the dominant species of bacteria so far isolated and proven to have plant growth promoting properties (Wang et al., 2021). In the present study, a total of 10 *Bacillus* spp. were isolated from soil in Kura local government area and CDA rice farms. Morphologically, almost all the isolates appeared to be milky white in colour and were mostly small and few large. As for shape, some were flat, irregular, and raised. All the isolates were sticky, and some are transparent while some were opaque. Microscopically, all the bacteria isolated were Gram positive, purple in colour, rod shaped and some appear singly, in chain or cluster and chained.

The biochemical results of the isolates were shown in **Table 1.** Isolates from different farmlands showed varying biochemical characteristics. The result of ammonia and hydrogen cyanide production by isolated *Bacillus* sp. is presented in **Table 2**. All the isolates displayed ammonia production with the exception of SA4 and SB3 while for hydrogen cyanide production, SA2, SA5 and SB3 were found not to secrete the compound. Several parameters are used to identify plant growth promoting bacteria. Ammonia and hydrogen cyanide production are among the most important features displayed by PGPR.

Table 1. Biochemical Characteristics of the isolates

Isolate	Catalase	Oxidase	Citrate	Urease	TSI	Spore test	Indole	Starch Hydrolysis	Motility
SA1	+	-	+	-	+	+	+	-	+
SA2	+	-	+	-	+	+	+	+	+
SA3	+	-	+	-	+	+	+	-	+
SA4	-	-	+	-	+	+	+	+	+
SA5	+	-	+	-	+	+	+	+	+
SB1	+	-	+	-	+	+	+	+	+
SB2	-	-	+	-	+	+	+	-	+
SB3	-	-	+	-	+	+	+	+	+
SB4	-	-	+	-	+	+	+	+	-
SB5	+	-	+	-	+	+	+	+	+

TSI= Triple Sugar Iron Test

Table 2. Screening results for NH_3 and HCN production of the Isolates

S/N	NH_3	HCN production
SA1	+	+
SA2	+	-
SA3	+	+
SA4	-	+
SA5	+	-
SB1	+	+
SB2	+	+
SB3	-	-
SB4	+	+
SB5	+	+

PGPR indirectly support crop development by producing ammonia, which causes the soil to become alkaline, preventing the growth of some pathogenic fungus, nitrobacteria, and inhibiting the germination of some pathogenic fungal spores (Mohanty et al., 2021). Directly, ammonia production can serve as a source of nitrogen to the plant which can enhance root and shoot growth (Abdelwahed et al., 2022). HCN is toxic to animals and bacteria. The compound blocks the electron transport chain, causing the cell's energy source to be cut off, resulting in the organism's death. It also interferes with the normal function of enzymes and natural receptors, notably the action of cytochrome oxidase. This is important to plants as the action can inhibit the growth of pathogenic organism surrounding the roots of the plants (Mazumdar et al., 2020). The three isolates were found to be the most ammonia-producing bacteria, indicating their potential as PGPR. Isolated bacteria in this investigation also produced HCN, indicating that these strains are potent PGPRs.

The isolates' HCN and NH3 production result is presented in **Table 2**. For the synthesis of ammonia, SA1, SA2, SA3, SA5, SB1, SB2, SB4, and SB5 are all positive, but for the production of HCN, SA1, SA3, SA4, SB1, SB2, SB4, and SB5 are all positive.

The quantitative result of Phosphate solubilisation presented as halo-zone in diameter (mm) by the isolates is shown in **Figure 1**. Phosphates occur as insoluble complexes especially in acidic soils which dominate the tropical countries. This makes it one of the unavailable mineral required by plants (Wang et al., 2021). Majority of

phosphate solubilising bacteria are from *Pseudomonas* species and the ability of bacteria to solubilise phosphate depends upon the release of organic acid by the microorganism (Pathak et al., 2019). The hydroxyl and carboxyl groups present in the organic acids normally quench cations and convert them to soluble forms. This is extremely important to plants as the process makes phosphates available for easy absorption by the roots of the plants (Tang et al., 2020). Based on the result presented, five of the 10 isolates had a visible halo zone. SA1 has the greatest value (28mm), followed by SA3 and SB1 (25mm). Luckily enough, these isolates also appeared to produce both ammonia and hydrogen cyanide. This made them potential candidates as PGPR.

The result of Zinc solubilisation by the isolates is presented in **Figure 1**. SA1 has the highest zone diameter, Followed by SA3 and SB1. Zinc is one of the micronutrient whose deficiency is not as a result of its availability but because of low solubility as a result of complex formation (Prasad et al., 2019). The element plays a vital role as cofactor for many enzymatic reactions in both plants and animals. However, certain species of growth promoting rhizobacteria can solubilise zinc from its complex and therefore makes it available for use by the plants (Pathak et al., 2019).

Production of indole 3 acetic acid is one of the prominent features of plant growth promoting rhizosphere. PGPR uses the production of IAA to manipulate the growth of their host through cell elongation, and organ development (Kumar, Patel, Meena, and Ramteke, 2019). The result of IAA production in (µg/ml) by the isolates is presented in **Figure 2**. From the results presented, SB1 has the highest IAA value of (99.61) followed by SA1 (99.17) and then SA3 (96.56) and were selected as the candidate isolate for further studies. The ability of a plant to acquire nutrient depends on its root and shoot length, IAA production by PGPR stimulate root and shoot elongation which eventually increases the capacity of the plant to absorb more nutrient leading to rapid growth of the plant (Kumar et al., 2019).

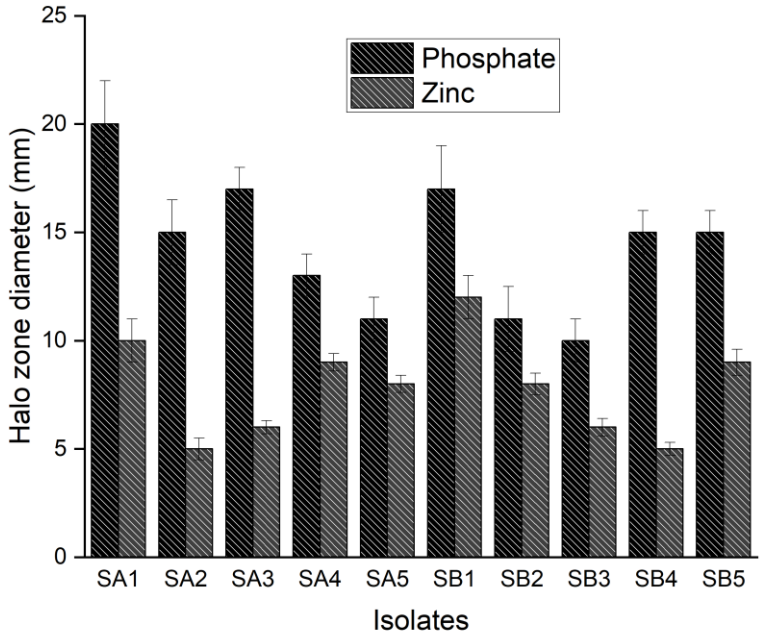

Fig. 1. Phosphate and Zinc solubilisation property of the isolates presented as halo zone diameter (mm). Values represent three independent determinations

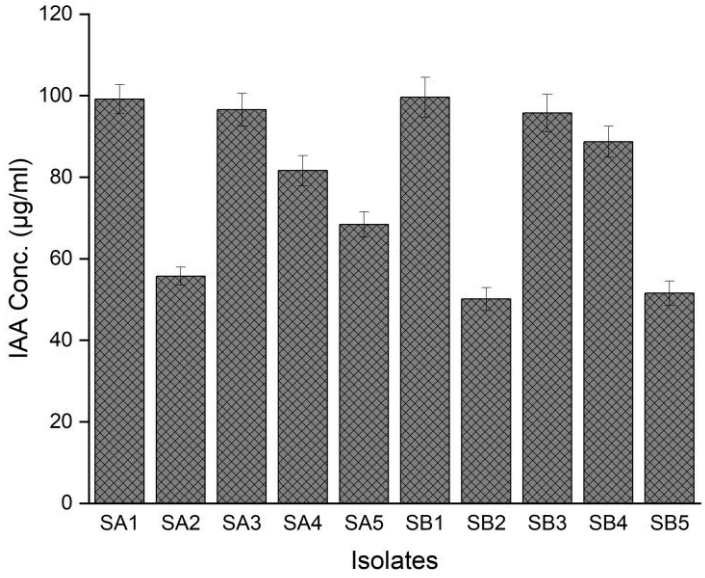

Fig. 2. Indole Acetic Acid (IAA) production by the Isolates. Values represent three independent determinations

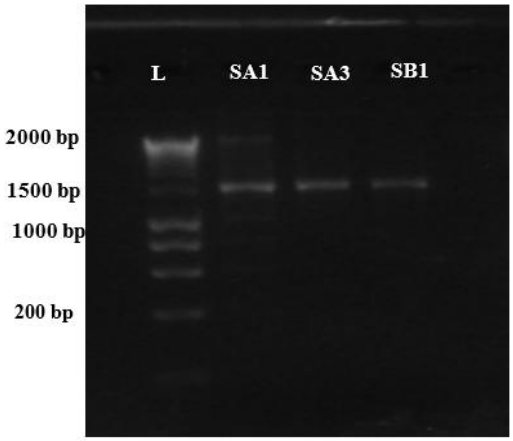

Fig. 3. Gel electrophoresis of the partial 16S rRNA of the PGPR isolates.

Having analyzed plant growth promoting properties displayed by the isolates, SA1, SA3 and SB1 appeared to relatively posses the features which will enable their usage as plant growth promoting Bacillus species. These bacteria were then chosen for further analysis. Gel electrophoresis was performed in order to visualize the amplified segment of the 16sRNA which was within the range of 1500 bp **Figure 3**. The 16S rRNA gene sequences of the bacteria isolated were compared with GenBank database using Blast Server at NCBI. Blast analysis of 16S rRNA sequences of the strains SA1, SA3 and SB1 revealed homology to *Bacillus subtilis* (99%), *Bacillus niacini*(98%), *Bacillus cereus* (99%), of the existing database of National Center of Bioinformatics, respectively (NCBI). Molecular phylogenetic studies using the neighbor joining method linked the identity of the obtained bacteria sequences to *Bacillus subtilis*, *Bacillus niacin* and *Bacillus cereus* (**Fig. 4**).

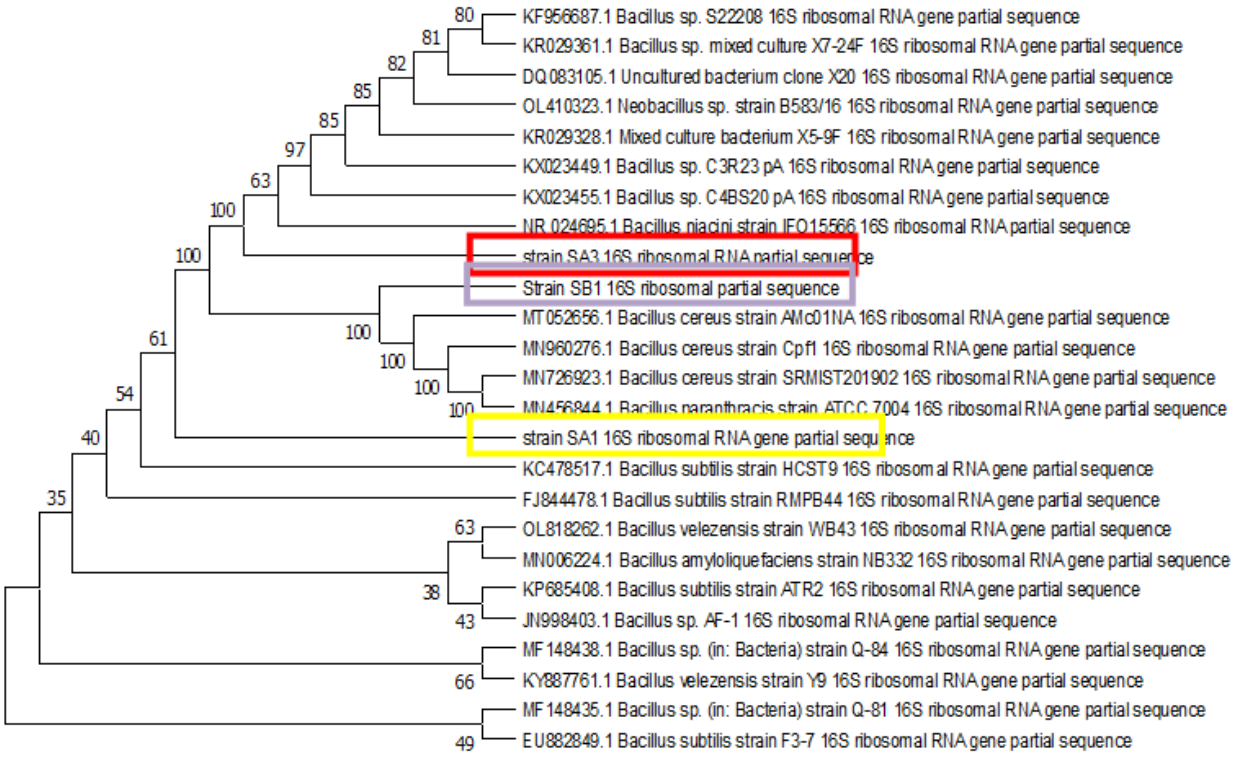

Fig. 4. Phylogenetic and Evolutionary relationships of taxa of strains. A rectangular rectangle denoted the strain's evolutionary position. Accession numbers are accompanied by the species' name. OM184294 (*Bacillus subtilis*) OM1842295 (*Bacillus niacini*) and OM184296 (*Bacillus cereus*)

Thus, these bacteria were tentatively assigned as *Bacillus subtilis strain* BUK_BCH_BTE_SA1, *Neobacillus niacini strain* BUK_BCH_BTE_SA3 and *Bacillus cereus* strain BUK_BCH_BTE_SB1 with accession numbers OM184294, OM1842295 and OM184296 respectively (**Fig. 4**). *Bacillus* spp. are the most available specie of rhizobacteria especially in tropical countries that were proven to dominate plant growth promoting specie of bacteria (Kashyap et al., 2019). Apart from promoting the growth through several mechanisms, *Bacillus* spp. were also known to reduce salinity stress in plants thereby prompting their utilization in salty environment (Kaloterakis et al., 2021).

Conclusions

In conclusion, ten bacterial strains were isolated from rhizosphere of two rice farms and screened for their plant growth promoting properties. Out of these ten, three isolates SB1, SB3 and SA proved to be the best from the results of the screening test. They were identified as *Bacillus subtilis*, *Bacillus niacini* and *Bacillus cereus* using 16SrRNA gene-based sequence. These three isolates have proven to be PGPRs. PGPRs are well known for their ability in plant growth promotion. Therefore, they could be used to enhance green agricultural revolution in crop production.

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. Abdelwahed S, Saadouli I, Kouidhi S, Masmoudi AS, Cherif A, Mnif W, Mosbah, A (2022). A new pioneer colorimetric micro-plate method for the estimation of ammonia production by plant growth promoting rhizobacteria (PGPR). Main Group Chemistry 21(1):55-68. DOI: 10.3233/MGC-210077.
2. Adnan N, Nordin SM, Anwar A (2020) Transition pathways for Malaysian paddy farmers to sustainable agricultural practices: An integrated exhibiting tactics to adopt Green fertilizer. Land use policy 90:104255. https://doi.org/10.1016/j.landusepol.2019.104255.
3. Aeron A, Khare E, Jha CK, Meena VS, Aziz SMA, Islam MT, Rajashekara H (2020) Revisiting the plant growth-promoting rhizobacteria: lessons from the past and objectives for the future. Archives of microbiology 202(4):665-676. doi:10.1007/s00203-019-01779-w.
4. Basu A, Prasad P, Das SN, Kalam S, Sayyed R, Reddy M, El Enshasy H (2021) Plant growth promoting rhizobacteria (PGPR) as green bioinoculants: recent developments, constraints, and prospects. Sustainability 13(3):1140. https://doi.org/10.3390/su13031140.
5. Garrity GM, Brenner DJ, Krieg N, Staley J, Manual BS (2005) Systematic bacteriology. The Proteobacteria, Part C: The Alpha-, Beta-, Delta-, and Epsiloproteobacteria, Bergey's Manual Trust, Department of Microbiology and Molecular Genetics. Springer USA P2.
6. Gouda S, Kerry RG, Das G, Paramithiotis S, Shin HS, Patra JK (2018) Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. Microbiological research 206:131-140. https://doi.org/10.1016/j.micres.2017.08.016.
7. Hashemnejad F, Barin M, Khezri M, Ghoosta Y, Hammer EC (2021) Isolation and identification of insoluble zinc-solubilising bacteria and evaluation of their ability to solubilise various zinc minerals. Journal of soil science and plant nutrition 21(3): 2501-2509. 10.1007/s42729-021-00540-x.
8. Kaloterakis N, van Delden SH, Hartley S, De Deyn GB (2021) Silicon application and plant growth promoting rhizobacteria consisting of six pure Bacillus species alleviate salinity stress in cucumber (*Cucumis sativus* L). Scientia Horticulturae 288: 110383. https://doi.org/10.1016/j.scienta.2021.110383.
9. Kashyap B K, Solanki MK, Pandey AK, Prabha, , Kumar P, Kumari B (2019) Bacillus as plant growth promoting rhizobacteria (PGPR): a promising green agriculture technology Plant health under biotic stress. Springer, pp. 219-236. DOI: 10.1007/978-981-13-6040-4_11.
10. Kumar A, Patel J, Meena VS, Ramteke P (2019) Plant growth-promoting rhizobacteria: strategies to improve abiotic stresses under sustainable agriculture. Journal of Plant Nutrition 42 (11-12):1402-1415. https://doi.org/10.1080/01904167.2019.1616757.

11. Mazumdar D, Saha SP, Ghosh S (2020) Isolation, screening and application of a potent PGPR for enhancing growth of Chickpea as affected by nitrogen level. International Journal of Vegetable Science 26(4):333-350. https://doi.org/10.1080/19315260.2019.1632401.
12. Mohanty P, Singh P K, Chakraborty D, Mishra S, Pattnaik R (2021) Insight into the role of PGPR in sustainable agriculture and environment. Frontiers in Sustainable Food Systems 5:667150. https://doi.org/10.3389/fsufs.2021.667150.
13. Mohite B. (2013) Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. Journal of soil science and plant nutrition 13(3):638-649. http://dx.doi.org/10.4067/S0718-95162013005000051.
14. Molnár K, Nyárádi II, Bíró-Janka B, Simó I, Bálint J, Domokos E. (2020) Preliminary Study of the Effect of Chemical and Organic Fertilizers on a Semi-Natural Grassland in Vlăhiţa, Harghita Mountains, Romania. Acta Biologica Marisiensis 3(2):56-65. DOI: 10.2478/abmj-2020-0011.
15. Mustafa S, Kabir S, Shabbir U, Batool R (2019) Plant growth promoting rhizobacteria in sustainable agriculture: from theoretical to pragmatic approach. Symbiosis 78(2):115-123. doi:10.1007/s13199-019-00602-w.
16. Nazir N, Kamili AN, Shah D (2018) Mechanism of plant growth promoting rhizobacteria (PGPR) in Enhancing plant growth: A review. Int. J. Manag. Technol. Eng 8:709-721.
17. Nicolopoulou-Stamati P, Maipas S, Kotampasi C, Stamatis P, and Hens L (2016) Chemical pesticides and human health: the urgent need for a new concept in agriculture. Frontiers in public health 4:148. https://doi.org/10.3389/fpubh.2016.00148.
18. Pathak D, Lone R, Khan S, Koul K (2019) Isolation, screening and molecular characterization of free-living bacteria of potato (*Solanum tuberosum* L.) and their interplay impact on growth and production of potato plant under mycorrhizal association. Scientia Horticulturae 252: 388-397. https://doi.org/10.1016/j.scienta.2019.02.072.
19. Péterfi O, Domokos E (2018) Mutualistic and Endophytic Microorganisms of: Description, Role and Use. Acta Biologica Marisiensis 1(2):5-21. DOI:https://doi.org/10.2478/abmj-2018-0009.
20. Prasad M, Srinivasan R, Chaudhary M, Choudhary M, Jat LK (2019) Plant growth promoting rhizobacteria (PGPR) for sustainable agriculture: perspectives and challenges. PGPR amelioration in sustainable agriculture 2:129-157. https://doi.org/10.1016/B978-0-12-815879-1.00007-0.
21. Rai PK, Singh M, Anand K, Saurabh S, Kaur T, Kour D, Kumar M (2020) Role and potential applications of plant growth-promoting rhizobacteria for sustainable agriculture New and Future Developments in Microbial Biotechnology and Bioengineering Elsevier, pp. 49-60. https://doi.org/10.1016/B978-0-12-820526-6.00004-X.
22. Rani L, Thapa K, Kanojia N, Sharma N, Singh S, Grewal AS, and Kaushal J. (2021) An extensive review on the consequences of chemical pesticides on human health and environment. Journal of Cleaner Production 283:124657. https://doi.org/10.1016/j.jclepro.2020.124657.

23. Sabarwal A, Kumar K, and Singh, RP (2018) Hazardous effects of chemical pesticides on human health–Cancer and other associated disorders. Environmental toxicology and pharmacology 63:103-114. https://doi.org/10.1016/j.etap.2018.08.018.
24. Sansinenea E (2019) Bacillus spp. as plant growth-promoting bacteria: Secondary metabolites of plant growth promoting rhizomicroorganisms. Springer pp225-237. DOI: 10.1007/978-981-13-5862-3_11.
25. Saxena A, Kumar M, Chakdar H, Anuroopa N, Bagyaraj D.(2020) *Bacillus* species in soil as a natural resource for plant health and nutrition. Journal of applied microbiology 128(6): 1583-1594. https://doi.org/10.1111/jam.14506.
26. Sehrawat A, Sindhu SS, Glick BR (2022) Hydrogen cyanide production by soil bacteria: Biological control of pests and promotion of plant growth in sustainable agriculture. Pedosphere 32(1): 15-38. https://doi.org/10.1016/S1002-0160(21)60058-9.
27. Sharma N, and Singhvi, R (2017) Effects of chemical fertilizers and pesticides on human health and environment: a review. International journal of agriculture, environment and biotechnology 10(6):675-680. 10.5958/2230-732X.2017.00083.3.
28. Tang A, Haruna AO, Majid NMA, and Jalloh MB (2020) Potential PGPR properties of cellulolytic, nitrogen-fixing, phosphate-solubilizing bacteria in rehabilitated tropical forest soil. Microorganisms 8(3):442. https://doi.org/10.3390/microorganisms8030442.
29. Wang Y, Peng S, Hua Q, Qiu C. Wu P, Liu, X, Lin X (2021) The long-term effects of using phosphate-solubilizing bacteria and photosynthetic bacteria as biofertilizers on peanut yield and soil bacteria community. Frontiers in Microbiology 3:12-18. 10.3389/fmicb.2021.693535.

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MEDICINAL PLANTS FROM THE FLORA OF ROMANIA BENEFICIAL IN OSTEOARTHRITIS AND RHEUMATIC ARTHRITIS

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Abstract: In this study, we focus on two arthritic diseases that affect a large part of the population, which cause inflammatory disorders of the joints and oxidative stress, which can cause certain degrees of disability. Arthritis is a chronic disease frequently encountered in the world's population. Osteoarthritis, and rheumatoid arthritis, autoimmune and inflammatory conditions, are two extensive forms of arthritis associated with pain, swelling, and stiffness in the joints and a low standard of life. Many drugs are used in their treatment, associated with some severe side effects and expensive prices. Today there are many studies carried out with extracts from medicinal plants, used in alternative therapy, and effective in these diseases. They are available for clinical use due to the active phytoconstituents that modulate inflammation and are antioxidants. In carrying out this study, electronic databases were screened: Science Direct, PubMed, and Google Scholar, trying to describe these medicinal plants, and elucidate their biological mechanisms of action. The most numerous references were found on the following plants: *Arctium lappa*, *Glycyrrhiza glabra*, *Nigella sativa*, *Urtica dioica*, etc. vary parts of plants are used such as different types of seeds, roots, leaves, fruit, bark, flowers, or even the whole plant.

Keywords: osteoarthritis; rheumatoid arthritis; medicinal plants; bioactive compounds

1. Introduction

Medicinal plants are an important natural wealth of the Earth. More than 20,000 plants from around the globe have medicinal or aromatic properties, even if only a tenth of them are currently used. The beginnings of their knowledge and use overlap with the beginnings of the rise of man, who turned to the healing properties of various plants, to relieve pain. The main remedies were almost entirely based on phytotherapy, so the foundations of rational medicine are found in folk medicine. This knowledge was transmitted, at first, orally and then in writing, in man's effort to prolong his life. In Romania, out of the c. 3,800 spontaneous plants (Sârbu et al., 2013), over 850 are used in traditional medicine. Out of these, about 300 are studied from a chemical-pharmaceutical point of view (Oroian, 2011).

One of the most common chronic articular diseases, osteoarthritis (OA) results from the destruction of articular cartilage and

subchondral bone (Kang et al., 2019). The major cause of OA is the disruption in the equilibrium between cartilage synthesis and degradation of joint cartilage. OA is considered an age-linked chronic condition with complex pathogenesis and without an effective and definitive method for its cure or, at least, management. Future studies need to sort out the mechanisms mediating the commencement and evolution of OA (Zhang et al., 2015; Gregori et al., 2018).

Considering that regular medication for OA does not have huge success and has a lot of side effects, in recent years, natural products that are known to have anti-inflammatory properties became broadly studied (Sharkey et al., 2021). Numerous herbs have been evaluated with well-documented studies. They proved to have encouraging potential for controlling pain and improving the evolution of arthritic conditions (Lindler et al., 2020; Anvari et al., 2020; Banic et al., 2021; etc.).

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease that has a progressive evolution. It can cause disability, and increase the risk of cardiovascular illness. RA is frequently related to increased levels of oxidative stress and inflammatory mediators (Lindler et al., 2020).

Our study gathers data about medicinal plant species present in Romanian flora, which can serve to further studies to establish the mechanism and action of these extracts in osteoarthritis and rheumatoid arthritis treatment.

2. Materials and methods

To collect the bibliographic data edited in the past 10 years (2012-2022), Science Direct, PubMed, and Google Scholar web search engines were used. We took into consideration the following keywords: medicinal herbs, bioactive compounds, osteoarthritis, rheumatoid arthritis, and a combination of them.

Information about the systematic classification, the part of the plant used, the dosage form, the major constituents of the plant extract, and the mechanism of action is presented. The parts of medicinal plants that are used are different types of seeds, roots, leaves, fruit, bark, flowers, or even the whole plant. The drug is noted in Latin (Oroian, 2011; Eșianu, 2016). The components of these plants can be used in different extract forms: Ethanolic, Methanolic, Hydroalcoholic, Aqueous extract, Petroleum ether extract, Hexane/ethanolic extract, etc.

The species name and the taxonomy are consistent with Euro+Med (2006+) and Sârbu et al. (2013). The species are listed in alphabetical order.

3. Results and discussions

Considering the bioactive compounds from herbal medicines used in the treatment of OA and RA 62 species that can be found in Romania's flora were identified, belonging to 38 families (**Table 1**). The most representative families are Asteraceae, Ranunculaceae, Solanaceae, etc. Among the medicinal plants selected, the most numerous data refer to the species: *Arctium lappa*, *Arnica montana*, *Glycyrrhiza glabra*, *Nigella sativa*, *Sesamum indicum*, *Symphytum officinale* (**Fig. 4**), *Urtica dioica*, *Coriandrum sativum*, etc. All these species have anti-inflammatory properties.

Leaves, roots, bark, fruits, seeds, flowers, or even the whole plant can contain active ingredients conferring medicinal properties to that plant. Many studies on herbs and their composition isolated the active compounds and documented their role in the biological effects. Among these, we mention phenolic acids, phenylpropanoid ester, triterpene glycosides, phthalides, flavonoids, alkaloids, triterpenoid

saponin, diterpene, and triterpene. New treatment opportunities for OA and RA patients can be obtained by having a good comprehension of the mechanism of action of the herbs and their compounds.

The main bioactive compounds with anti-OA and anti-RA activities in the medicinal plants identified in Romania's flora are flavonoids, essential oils, saponins, mucilages, alkaloids, iridoids, phenolic glycosides, etc.

Flavonoids (Quercetin, Hesperidin, Baicalin, Aglycone, Gentakwanin Hydroxygenkwanin, Luteolin, Apigenin, Liquiritin, Kaempferol, etc.) have antioxidant, anti-inflammatory, anti-edematous and immunomodulatory properties. These effects are explained by the property of flavonoids to inhibit the chemical mediators responsible for inflammation. Comparing the flavonoids to other active phytocompounds proves they have broader action and relatively lower toxicity. (Eșianu, 2016; Oroian, 2011) Among the plant drugs rich in flavonoids, we mention: Rutae herba, Sambuci flos, Betulae folium, etc. (**Table 1**).

Alkaloids are very widespread in the world of cormophytes. More than 12,000 alkaloids have been identified to date, with an estimated 10-15% of cormophytes containing alkaloids. (Sinomenine, Nicotine, Berberine, Koumine etc.). They act on the cytokines IL-6, IL-12, IL-1α, TNF-α, IL-1β, and IL-10 by regulating their level and have especially immunomodulatory effect. Among the plant drugs rich in alkaloids we mention Ephedrae herba Colchici bulbus et semen, Capsici fructus (contain non-heterocyclic alkaloids), Chelidonii herba, Chelidonii radix, Boraginis semen, Symphyti radix (pyrrolizidine alkaloids) (Eșianu, 2016; Oroian, 2011)

Triterpene saponins are widespread in the plant world, being concentrated in cell vacuoles. Saponins contained in plant drugs such as Liquiritiae radix, Equiseti herba, Hedere folium, are: asiaticoside, araloside A, medecassoside, pedunculoside. These have antirheumatic effects acting in fibroblast-like synoviocytes having immunostimulatory properties (Eșianu, 2016). Similarly, Mucilages (heteropolysaccharides) are also present in herbal drugs with anti-inflammatory effects in arthritic diseases. Among the herbal drugs rich in mucilage we mention Althaeae radix et folium, Lini semen, Plantaginis folium, etc.

Most of the studies analyzed were made in vitro and highlighted the anti-inflammatory effect of the medicinal herb extracts. Moreover, the antioxidant properties of biological compounds proved to have an impact on the treatment of OA and RA.

Several plant extracts presented in this paper proved to be good assets for pain reduction and improved mobility. The risk of side effects in arthritic subjects turned out to be lower. These results warrant further investigation (Choudhary et al., 2015; Ulbricht et al., 2014; Dragoș et al., 2017; etc.)

In the checklist of medicinal plants, they are presented in alphabetical order (**Table 1**). The data recorded provide us with information on scientific name, family, common name, the part(s) used, the form of use, major phytoconstituents, therapeutic activity, mechanism of action, and references.

Most of the plant species listed in **table 1** are from the native flora of Romania, but we have also recorded a few cultivated species: *Allium cepa, Allium sativum, Althaea rosea, Capsicum annuum, Coriandrum sativum, Elaeagnus angustifolia* (**Fig. 1**), *Linum usitatissimum, Lonicera japonica, Ocimum basilicum, Ruta graveolens, Sesamum indicum*, etc. which have been intensively studied from a pharmacological point of view, being then included into the composition of some medicines.

Fig. 1. *Elaeagnus angustifolia*

Fig. 2. *Ricinus communis*

Romania's flora is rich in medicinal plants used in arthritis. Thus we mention some of them known to have strong anti-inflammatory or analgesic effects. Thus, *Nigella sativa* - Black cumin (Nigellae semen) is considered a potential candidate in the management of arthritis, *Reynoutria japonica* - Japanese knotweed (Reynoutriae radix) a good anti-inflammatory or *Glycyrrhiza glabra* - Licorice (Liquiritiae radix - officinal in Ph. Eur. and FR X) containing flavonoids, flavones, flavonals, isoflavones, etc. with antioxidant, antibacterial and anti-inflammatory activities, *Arnica montana* - Mountain horsetail (Arnicae flos-officinal in Ph. Eur.) whose dominant active principles: phenolic and flavonoid compounds would determine the anti-arthritic efficiency, *Elaeagnus angustifolia* - Smelly willow (Elaeagni fructus) of whose extract contains Kaemferol with particular analgesic and anti-inflammatory action. We also mention the species: *Borago officinalis* – Lamb's tongue (Boraginis semen - officinal in Ph.Eur.) (Boraginis herba) whose oil contains Gamma-linoleic acid with an anti-inflammatory effect or *Symphytum officinale*- Comfrey (Symphyti radix et folium) whose extract has analgesic and anti-inflammatory properties due to allantoin, rosmarinic acid and other derivatives of hydroxycinnamic acid and mucopolysaccharides (due to the content of pyrrolizidine alkaloids, with a hepatotoxic and carcinogenic effect, internal use is not recommended, and external use is limited), etc.

In the flora of our country, there are also plants whose extracts contain toxic active principles that have good analgesic or anti-inflammatory effects (which we did not mention in the paper). We mention *Cannabis sativa* - Hemp (Cannabis herba) whose main chemical component is a resin containing cannabidiol with anti-arthritic effects through anti-inflammatory and immunosuppressive action.

Papaver somniferum – Garden poppy (Papaveri imaturi fructus/opium - officinal in Ph.Eur. and FR X) considered to be a very good analgesic (morphine, codeine, and opium abuse gives rise to drug addictions, creates mental and physical dependence).

In this study, we also have a group of plants that are used in the treatment of arthritis but which are also toxic, so their extracts should only be used under medical supervision. Among these we mention *Colchicum autumnale* - The autumn crocus (Colchici bulbus et semen), which contains colchicine and has anti-arthritic and anti-inflammatory effects. Colchicine also has a mutagenic action, which is why it has uses in genetic engineering, as a plant polyploidizer. Toxicity can pass from animals, through milk, and meat, to consumers.

That is why the pastures must be cleared of these plants.

Ruta graveolens - Rue (Rutae herba) - the polyphenolic fraction of the extract from the aerial parts have an anti-arthritic action, but at the same time it is a dangerous plant because it causes metrorrhagia and gastroenteritis; recently, its spasmolytic properties have also been highlighted.

Ricinus communis (**Fig. 2**) - Castor Bean (Ricini semen - officinal in Ph. Eur. and FR X), the seeds contain polyphenols and flavonoids with anti-inflammatory action but also contain ricin, a highly toxic substance.

We also mention the presence, of some invasive medicinal species in our country, with various origins, such as *Oenothera biennis* (North America), *Portulaca oleracea* (Asia), *Reynoutria japonica* (Eastern Asia) (**Fig. 3**), *Xanthium strumarium* (North America), etc., whose chemical composition is rich in active compounds with beneficial effects in the treatment of certain diseases. For example, *Reynoutria japonica* species with a devastating impact on the biodiversity of the habitats where it occur, stands out for its special chemical composition, because the root and leaf extracts contain active principles with special therapeutic potential. In vivo and in vitro studies determined the identification of the main compounds: resveratrol, emodin, and polydatin which can exert special therapeutic effects. That is why additional pharmacological research would be needed to highlight the benefits of this invasive species, and new experiments and human clinical evidence are needed. Recently Nawrot-Hadzik et al. (2021) reported the effects of vanicoside in particular on the inhibition of SARS-CoV-2 Mpro cells.

Joint inflammation is one of the symptoms of arthritis. The onset and evolution of it cause the destruction of the articular cartilage and stimulates the synovial membrane. A notable role in this condition plays oxidative stress, as well as the anti-inflammatory response triggered by the immune system. T cells are activated and inflammatory mediators such as cytokines and chemokines are influenced (Katturajan and Sabina, 2021).

Fig. 3. *Reynoutria japonica*

Fig. 4. *Symphytum officinale* L.

The mechanism of action of medicinal plants with anti-arthritic effect acts by influencing the signaling pathways (NF-kB, RANKL, and PI3K/Akt).

They regulate pro-inflammatory and pro-catabolic mediators such as cytokines, PGE2, MMP, ROS, and apoptotic proteins These activities can help improve OA and RA joint pain, inflammation, swelling, structure, and function with minimal adverse effects (Lindler et al., 2020).

Numerous studies on the human and animal models certify that the main mechanism of action in the alleviation of RA symptoms includes inhibition of the expression of NF-κB, IL-1β, TNF-α, IL-6, IL-8, IL-17, IL-23, chemokines, TGF-β, RANKL, RANK, iNOS, arginase, COX-2, VEGFA, VEGFR, NFATC1, and TRAP in the synoviocytes. Reduced ROS, NO, MDA, carbonyl groups, and PGE2 in the joint fluid elevated the expression of PPARα/γ. A notable role in ameliorating rheumatoid arthritis etiology has antioxidant and anti-inflammatory molecules (Arunsi et al., 2022).

The effect of medicinal plants listed in this study generally follows the validated mechanism. Most of the studies refer to the downregulation of inflammatory factors and cytokines. Other plants have antiarthritic use due to the antioxidative properties of their compounds.

Most studies have been conducted in vitro, often in mice and very rarely in human subjects.

Conclusions

In this review, we have compiled and analyzed 62 medicinal species which have active compounds with anti-OA activities. All studies demonstrate the anti-inflammatory effects of extracts containing one or more secondary metabolites and suggest their anti-arthritic efficacy.

Because the pathogenesis of rheumatoid arthritis and its immune mechanism has not been fully understood, it still requires further studies.

The potential of plant raw material is enormous therefore there are great prospects in obtaining new, more effective drugs that can become an alternative treatment for arthritis. In addition, these herbs have proven effects for a long ago and they don’t have severe side effects.

We reviewed only a small sample of herbs that modulate inflammation and are available for clinical use. The aim was to point out that they differ notably from all anti-inflammatory drugs with regard to actions and security and to provide insight into their clinical use. Not explored in this paper, but of greatest importance is the inclusion of increased amounts of plants in the diet, meaning fruits, vegetables, whole grains, nuts, herbs, and spices. These will provide abundant inflammation-modulating compounds that go a long way. in calming an inflammatory state.

Finally, we have come to the conclusion that today, the return and interest in the use of medicinal plants in the treatment of arthritis is increasing. The special effort of specialists, from several fields of study, in deciphering the factors that cause these chronic inflammatory disorders that cause serious histological alterations was found. Thus, botanists (whose purpose is to identify medicinal species, their quantitative evaluation for rational exploitation, etc.), biochemists (with a role in identifying the chemical composition of drugs, the active principles and their method of extraction, etc.), pharmacologists who have the purpose to identify the mechanisms of action of plant extracts, mechanisms that have often proven particularly effective in various in vitro or animal studies. However, today there is a dearth of human clinical evidence. It is recommended that in the coming years, special

attention be paid to clinical investigations so that animal studies can be translated into humans.

It would be very interesting and useful through future studies to determine the possibility of using some compounds with anti-inflammatory and regenerative properties, directly into the joint. This way achieves much higher concentrations in the affected joint and implicitly a stronger effect without passing through the systemic circulation; the side effects are much smaller due to the fact that the substances are difficult to absorb from the joint.

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. Abolhassanzadeh Z, Aflaki E, Yousefi G, Mohagheghzadeh A (2015) Randomized Clinical Trial of Peganum Oil for Knee Osteoarthritis. Journal of Evidence-Based Complementary & Alternative Medicine, 20(2):126-131. doi:10.1177/2156587214566867
2. Allahmoradi E, Taghiloo S, Omrani-Nava V, Shobeir SS, Tehrani M, Ebrahimzadeh MA, Asgarian-Omran H (2018) Anti-inflammatory effects of the Portulaca oleracea hydroalcholic extract on human peripheral blood mononuclear cells. Medical journal of the Islamic Republic of Iran, 32, 80. https://doi.org/10.14196/mjiri.32.80
3. Anvari M, Dortaj H, Hashemibeni B, Pourentezari M (2020) Application of Some Herbal Medicine Used for the Treatment of Osteoarthritis and Chondrogenesis. Trad Integr Med. 5(3):126-149
4. Arjumand S, Shahzad M, Shabbir A, Yousaf MZ (2019) Thymoquinone attenuates rheumatoid arthritis by downregulating TLR2, TLR4, TNF-α, IL-1, and NFκB expression levels. Biomedicine & pharmacotherapy, 111, 958–963. https://doi.org/10.1016/j.biopha.2019.01.006
5. Arunsi UO, Chioma OE, Etusim PE., Owumi SE (2022) Indigenous Nigeria medicinal herbal remedies: A potential source for therapeutic against rheumatoid arthritis. Experimental biology and medicine (Maywood, N.J.), 247(13), 1148–1178. https://doi.org/10.1177/15353702221102901
6. Askari A, Ravansalar SA, Naghizadeh MM, Mosavat SH, Khodadoost M, Jazani AM, Hashempur MH (2019) The efficacy of topical sesame oil in patients with knee osteoarthritis: A randomized double-blinded active-controlled non-inferiority clinical trial. Complementary therapies in medicine, 47, 102183. https://doi.org/10.1016/j.ctim.2019.08.017
7. Banik B, Das S, Das M. Kumar (2021) Medicinal Plants with Potent Anti-inflammatory and Anti-arthritic Properties found in Eastern Parts of the Himalaya: An Ethnomedicinal Review. Pharmacognosy Reviews, vol. 14, no. 28, pp. 121-137.
8. Bonaterra GA, Heinrich EU, Kelber O, Weiser D, Metz J, Kinscherf R (2010) Anti-inflammatory effects of the willow bark extract STW 33-I (Proaktiv(®)) in LPS-activated human monocytes and differentiated macrophages. Phytomedicine: international journal of phytotherapy and phytopharmacology, 17(14), 1106–1113.

https://doi.org/10.1016/j.phymed.2010.03.022
9. Choudhary M, Kumar V, Malhotra H, Singh S (2015) Medicinal plants with potential anti-arthritic activity. Journal of intercultural ethnopharmacology, 4(2), 147–179. https://doi.org/10.5455/jice.20150313021918
10. Council of Europe., E.D. for the Q. of M.& H. European Directorate for the Quality of Medicines & Health Care, *European Pharmacopoeia*; Council of Europe: Strasbourg, 2014; ISBN 978-92-871-7525-0.
11. Cucu AA, Baci GM, Dezsi Ş, Nap ME., Beteg FI, Bonta V, Bobiş O, Caprio E, Dezmirean DS (2021) New Approaches on Japanese Knotweed (Fallopia japonica) Bioactive Compounds and Their Potential of Pharmacological and Beekeeping Activities: Challenges and Future Directions. Plants, 10(12), 2621. MDPI AG. Retrieved from http://dx.doi.org/10.3390/plants10122621
12. Dragoş D, Gilca M, Gaman L, Vlad A, Iosif L, Stoian I, Lupescu O (2017) Phytomedicine in Joint Disorders. *Nutrients*, *9*(1), 70. https://doi.org/10.3390/nu9010070
13. Dursus C (2018) La gemmothérapie appliquée aux pathologies ostéo-articulaires fréquemment rencontrées à l'officine. Sciences pharmaceutiques. ⟨dumas-01714565⟩. https://dumas.ccsd.cnrs.fr/dumas-01714565/document
14. El-Sheikh SMA, El-Alim AAFA, Galal AAAG, El-Sayed RG, Al-Gabri NA (2019) Rheumatoid arthritis: Herbal medicines as alternative source to alleviate its symptoms and current animal models. Vet. Sci. 7(s2): 71-78. DOI:http://dx.doi.org/10.17582/journal.aavs/2019/7.s2.71.78. ISSN (Online): 2307-8316; ISSN (Print): 2309-3331
15. Farmacopeea Romana - editia a X-a, Edit. Medicala, 2021
16. Eşianu S (2016) Manual de Farmacognozie, vol. I-II, Edit. University Press UMFST Târgu Mureș.
17. Euro+Med 2006+ [continuously updated]: Euro+Med PlantBase - the information resource for Euro-Mediterranean plant diversity. – Published at http://www.europlusmed.org [Accessed October 2022].
18. Farzaei MH, Farzaei F, Abdollahi M, Abbasabadi Z, Abdolghaffari AH, Mehraban B (2016). A mechanistic review on medicinal plants used for rheumatoid arthritis in traditional Persian medicine. The Journal of pharmacy and pharmacology, 68(10), 1233–1248. https://doi.org/10.1111/jphp.12606
19. Gandhi Y, Kumar R, Grewal J, Rawat H, Mishra SK, Kumar V, Shakya SK, Jain V, Babu G, Sharma P, Singh A (2022) Advances in anti-inflammatory medicinal plants and phytochemicals in the management of arthritis: A comprehensive review. Food chemistry advances, 1, 100085. doi: 10.1016/j.focha.2022.100085
20. Gautam RK, Roy K, Thapa G, Arora D, Parashar S, Gurung B, Deb LC (2020) Perspective of Plant Medicine in Therapy of Rheumatoid Arthritis. Indian Journal of Pharmaceutical Sciences, 82, 741-765.
21. Ghasemian M, Owlia S, Owlia MB (2016) Review of Anti-Inflammatory Herbal Medicines. Advances in pharmacological sciences, 2016, 9130979. https://doi.org/10.1155/2016/9130979
22. Goswami NG, Koli M, Singh A, Giri D (2022) Urtica dioica: An undervalued herb a comprehensive review. Journal of

Pharmacognosy and Phytochemistry, 11(3), 169-173.

23. Gregori D, Giacovelli G, Minto C, Barbetta B, Gualtieri F, Azzolina D, Vaghi P, Rovati LC (2018) Association of Pharmacological Treatments With Long-term Pain Control in Patients With Knee Osteoarthritis: A Systematic Review and Meta-analysis. JAMA, 320(24), 2564–2579. https://doi.org/10.1001/jama.2018.19319
24. Henrotin Y, Mobasheri A (2018) Natural Products for Promoting Joint Health and Managing Osteoarthritis. Current Rheumatology Reports, 20(11). doi:10.1007/s11926-018-0782-9
25. Hougee S, Faber J, Sanders A, Berg WB, Garssen J, Smit HF, Hoijer MA (2006) Selective Inhibition of COX-2 by a Standardized CO2 Extract of *Humulus lupulus* in Vitro and its Activity in a Mouse Model of Zymosan-Induced Arthritis. Planta Medica, 72(3), 228–233 doi:10.1055/s-2005-916212
26. Hussain A, Aslam B, Muhammad F, Faisal MN, Kousar S, Mushtaq A, Bari MU (2021) Anti-arthritic activity of *Ricinus communis* L. and *Withania somnifera* L. extracts in adjuvant-induced arthritic rats *via* modulating inflammatory mediators and subsiding oxidative stress. Iranian journal of basic medical sciences, 24(7), 951–961. https://doi.org/10.22038/ijbms.2021.55145.12355
27. Jiang X, Qu Q, Li M, Miao S, Li X, Cai W (2014) Horsetail mixture on rheumatoid arthritis and its regulation on TNF-α and IL-10. Pakistan journal of pharmaceutical sciences, 27(6 Suppl), 2019–2023.
28. Kaithwas G, Majumdar DK (2010) Therapeutic effect of Linum usitatissimum (flaxseed/linseed) fixed oil on acute and chronic arthritic models in albino rats. Inflammopharmacology, 18(3), 127–136. https://doi.org/10.1007/s10787-010-0033-9
29. Kang YH, Lee HJ, Lee CJ, Park JS (2019) Natural Products as Sources of Novel Drug Candidates for the Pharmacological Management of Osteoarthritis: A Narrative Review. Biomolecules & therapeutics, 27(6), 503–513. https://doi.org/10.4062/biomolther.2019.139
30. Katturajan R, Sabina EP (2021) Joint Inflammation: Insights of Osteoarthritis, Gouty and Rheumatoid Arthritis and its Prevalence, Mechanism, Medications and Remedies. Indian Journal of Pharmaceutical Sciences, 83(5), 886-898.
31. Kis B, Avram S, Pavel IZ, Lombrea A, Buda V, Dehelean CA, Soica C, Yerer MB, Bojin F, Folescu R, Danciu C (2020) Recent Advances Regarding the Phytochemical and Therapeutic Uses of Populus nigra L. Buds. Plants (Basel, Switzerland), 9(11), 1464. https://doi.org/10.3390/plants9111464
32. Lin B, Zhao Y, Han P, Yue W, Ma XQ, Rahman K, Zheng CJ, Qin LP, Han T (2014) Anti-arthritic activity of Xanthium strumarium L. extract on complete Freund′s adjuvant induced arthritis in rats. Journal of ethnopharmacology, 155(1), 248–255. https://doi.org/10.1016/j.jep.2014.05.023
33. Lindler BN, Long KE, Taylor NA, Lei W (2020) Use of Herbal Medications for Treatment of Osteoarthritis and Rheumatoid Arthritis. Medicines (Basel, Switzerland), 7(11), 67. https://doi.org/10.3390/medicines7110067
34. Midhun Kumar GH, Shanmuga Priyan Arunachalam P, Elavarasan K (2020) Rheumatoid Arthritis: Treatment and Pharmacological Therapies. Medico Legal Update, 20(4), 2336–2339. https://doi.org/10.37506/mlu.v20i4.2195
35. Musco N, Vassalotti G, Mastellone V, Cortese L, della Rocca G, Molinari ML, Calabrò S, Tudisco R, Cutrignelli MI,

Lombardi P (2019) Effects of a nutritional supplement in dogs affected by osteoarthritis. Veterinary Medicine and Science. doi:10.1002/vms3.182

36. Nadipelly Dr Jagan, Jayasree T., Rao Balida, Kumar K, Kumar S (2012) Evaluation of the Anti-nociceptive and Anti-inflammatory Activities of the Pet: Ether Extract of Portulaca Oleracea (Linn.). Journal of Clinical and Diagnostic Research. 6. 226-230.
37. Nawrot-Hadzik I, Zmudzinski M, Matkowski A, Preissner R, Kęsik-Brodacka M, Hadzik J, Drag M, Abel R. (2021) *Reynoutria* Rhizomes as a Natural Source of SARS-CoV-2 Mpro Inhibitors-Molecular Docking and In Vitro Study. Pharmaceuticals (Basel, Switzerland), 14(8), 742. https://doi.org/10.3390/ph14080742
38. Oroian S (2011) Botanică Farmaceutică, Edit. University Press, Târgu Mureș
39. Oroian S, Sămărghiţan M, Coşarcă S, Hiriţiu M, Oroian F, Tanase C (2019) Botanical Survey of Medicinal Plants Used in the Traditional Treatment of Human Disease in Montain Hay Meadows from Gurghiului Mountains. Acta Biologica Marisiensis, 2(1) 38-46. https://doi.org/10.2478/abmj-2019-0005
40. Panahi Y, Alishiri GH, Bayat N, Hosseini SM, Sahebkar A (2016) Efficacy of Elaeagnus Angustifolia extract in the treatment of knee osteoarthritis: a randomized controlled trial. *EXCLI journal*, *15*, 203–210. https://doi.org/10.17179/excli2015-639
41. Park YJ, Cho YR, Oh JS, Ahn EK (2017) Effects of Tribulus terrestris on monosodium iodoacetate-induced osteoarthritis pain in rats. Molecular medicine reports, *16*(4), 5303–5311. https://doi.org/10.3892/mmr.2017.7296
42. Ra HJ, Lee HJ, Jo HS, Nam DC, Lee YB, Kang BH, Moon DK, Kim DH, Lee CJ, Hwang, SC (2017) Betulin suppressed interleukin-1β-induced gene expression, secretion and proteolytic activity of matrix metalloproteinase in cultured articular chondrocytes and production of matrix metalloproteinase in the knee joint of rat. The Korean journal of physiology & pharmacology: official journal of the Korean Physiological Society and the Korean Society of Pharmacology, 21(1), 19–26. https://doi.org/10.4196/kjpp.2017.21.1.19
43. Rai A (2013) The Antiinflammatory and Antiarthritic Properties of Ethanol Extract of Hedera helix. Indian journal of pharmaceutical sciences, 75(1), 99–102. https://doi.org/10.4103/0250-474X.113537
44. Reddy VJ, Rao PG, Lakshmi GR. A review on antiarthritic activity of some medicinal plants. J Glob Trends Pharm Sci. 2014;5(4):2061-73.
45. Sahu D, Raghav SK, Gautam H, Das HR (2015) A novel coumarin derivative, 8-methoxy chromen-2-one alleviates collagen induced arthritis by down regulating nitric oxide, NFκB and proinflammatory cytokines. International immunopharmacology, 29(2), 891–900. https://doi.org/10.1016/j.intimp.2015.08.012
46. Saleem S, Khan R, Kazmi I, Afzal M (2019) Medicinal Plants in the Treatment of Arthritis. Plant and Human Health, Volume 3, 101–137. doi:10.1007/978-3-030-04408-4_6.
47. Sârbu I, Ştefan N, Oprea A (2013) Plante vasculare din România, determinator ilustrat de teren. Bucureşti: Edit. VictorBVictor, 1317 pp.
48. Seigner J, Junker-Samek M, Plaza A, D'Urso G, Masullo M, Piacente S, Holper-Schichl Y M, de Martin R (2019) A

Symphytum officinale Root Extract Exerts Anti-inflammatory Properties by Affecting Two Distinct Steps of NF-κB Signaling. Frontiers in pharmacology, 10, 289. https://doi.org/10.3389/fphar.2019.00289

49. Seigner J, Junker-Samek M, Plaza A, D'Urso G, Masullo M, Piacente S, Holper-Schichl YM, de Martin R (2019) A Symphytum officinale Root Extract Exerts Anti-inflammatory Properties by Affecting Two Distinct Steps of NF-κB Signaling. Frontiers in pharmacology, 10, 289. https://doi.org/10.3389/fphar.2019.00289
50. Sharkey P, Zubin Shah, Gross M, Meade T, Davidoff S, Shohat N, Parvizi J (2021) The Effect of a Natural Oral Nutritional Supplement on the Level of Intra-Articular Inflammatory Mediators in Patients with Osteoarthritis of the Knee. Journal of Orthopaedic Experience & Innovation, April
51. Sharma S, Arif M, Nirala RK, Gupta R, Thakur SC (2016). Cumulative therapeutic effects of phytochemicals in Arnica montana flower extract alleviated collagen-induced arthritis: inhibition of both pro-inflammatory mediators and oxidative stress. Journal of the science of food and agriculture, 96(5), 1500–1510. https://doi.org/10.1002/jsfa.7252
52. Singh S, Singh TG, Mahajan K, Dhiman S (2020) Medicinal plants used against various inflammatory biomarkers for the management of rheumatoid arthritis. The Journal of pharmacy and pharmacology, 72(10), 1306–1327
. https://doi.org/10.1111/jphp.13326
53. Suszko A, Obmińska-Mrukowicz B (2017) Effects of polysaccharide fractions isolated from *Caltha palustris* L. on the activity of phagocytic cells & humoral immune response in mice with collagen-induced arthritis: A comparison with methotrexate. The Indian journal of medical research, 145(2), 229–236.
https://doi.org/10.4103/ijmr.IJMR_704_14
54. Triastuti A, Pradana DA, Saputra DE, Lianika N, Wicaksono HR, Anisari TD, Widyarini S (2021) Anti-rheumatoid activity of a hexane-insoluble fraction from *Plantago major* in female Wistar rats induced by Complete Freund's Adjuvant. Journal of traditional and complementary medicine, 12(3), 219–224.
https://doi.org/10.1016/j.jtcme.2021.07.006
55. Tsai SW, Lin CC, Lin SC, Wang SP, Yang DH (2019) Isorhamnetin ameliorates inflammatory responses and articular cartilage damage in the rats of monosodium iodoacetate-induced osteoarthritis. Immunopharmacology and immunotoxicology, 41(4), 504–512.
https://doi.org/10.1080/08923973.2019.1641723
56. Tsiogkas SG, Grammatikopoulou MG, Gkiouras K, Zafiriou E, Papadopoulos I, Liaskos C, Dardiotis E, Sakkas LI, Bogdanos DP (2021) Effect of *Crocus sativus* (Saffron) Intake on Top of Standard Treatment, on Disease Outcomes and Comorbidities in Patients with Rheumatic Diseases: Synthesis without Meta-Analysis (SWiM) and Level of Adherence to the CONSORT Statement for Randomized Controlled Trials Delivering Herbal Medicine Interventions. Nutrients, 13(12), 4274. https://doi.org/10.3390/nu13124274
57. Ulbricht C, Windsor RC (2014) An Evidence-Based Systematic Review of Black cohosh (Cimicifuga racemosa, Actaea racemosa) by the Natural Standard Research Collaboration. Journal of Dietary Supplements, 12(3), 265–358.
doi:10.3109/19390211.2014.946731
58. Villeneuve E, (2017) Place de la phytothérapie dans la prise en charge des douleurs articulaires chroniques à l'officine,

thèse d'exercice, Limoges, Université de Limoges. Disponible sur http://aurore.unilim.fr/ori-oai-search/notice/view/unilim-ori-76076

59. Xia X, May BH, Zhang AL, Guo X, Lu C, Xue CC, Huang Q (2020) Chinese Herbal Medicines for Rheumatoid Arthritis: Text-Mining the Classical Literature for Potentially Effective Natural Products. Evidence-based complementary and alternative medicine: eCAM, 2020, 7531967. https://doi.org/10.1155/2020/7531967
60. Yang CLH, Or TCT, Ho MHK, Lau ASY (2012) Scientific Basis of Botanical Medicine as Alternative Remedies for Rheumatoid Arthritis. Clinical Reviews in Allergy & Immunology, 44(3), 284–300. doi:10.1007/s12016-012-8329-8
61. Yang J, Sun Y, Cao F, Yang B, Kuang H (2022) Natural Products from *Physalis alkekengi* L. var. *franchetii* (Mast.) Makino: A Review on Their Structural Analysis, Quality Control, Pharmacology, and Pharmacokinetics. Molecules (Basel, Switzerland), 27(3), 695. https://doi.org/10.3390/molecules27030695
62. Zang Y (2020) Pharmacological Activities of Coumarin Compounds in Licorice: A Review. Natural Product Communications, 15(9). doi:10.1177/1934578X20953954
63. Zhang D, Huang B, Xiong C, Yue Z (2015) Pinocembrin inhibits matrix metalloproteinase expression in chondrocytes. IUBMB Life. 67:36–41. DOI: 10.1002/iub.1343
64. Zhao L, Wang L, Di SN, Xu Q, Ren QC, Chen SZ, Huang N, Jia D, Shen XF (2018). Steroidal alkaloid solanine A from Solanum nigrum Linn. exhibits anti-inflammatory activity in lipopolysaccharide/interferon γ-activated murine macrophages and animal models of inflammation. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie*, *105*, 606–615. https://doi.org/10.1016/j.biopha.2018.06.019

Table 1. Characteristics of medicinal plants beneficial in the treatment of osteoarthritis

Botanical name English name Family	Drugs	Dosage form	Uses	Major phytoconstituents	Effects	Mechanism of Action/	References
Actaea racemosa Black cohosh Ranunculaceae	Cimicifugae radix	Extract	Arthritis	Actein, cimigoside, steroidal terpenes, and 27-deoxyactein	Anti-inflammatory Anti-arthritic Reduce edema, bone resorption, reduce chronic arthritis pain	inhibits mRNA of cytokines IL-4, IL-5 and TNF-α	Saleem et al., 2019 Choudhary et al., 2015 Ulbricht et al., 2014 Yang et al., 2012
Allium cepa Onion Amaryllidaceae	Allii cepae bulbus	Paste	Rheumatoid Arthritis	Quercetin	Anti-arthritic	Lessen the purinergic system (E-NTPDase and E-ADA activities) and the quantities of IFN-gamma and IL-4. Depletion in leukocytosis and immune expression of TNF-α, IL-17 in the synovium. Restrain the production of proinflammatory cytokines (IL-1β, IL-6, and IL-8)	Arunsi et al., 2022 Tsai et al., 2019
Allium sativum Garlic Amaryllidaceae	Allii sativi bulbus et semen	Oil	Anti-arthritic	Diallyl sulfide	Anti-arthritic	Reduces paw edema and the levels of CRP. Suppresses the expression of TNF-α, IL-1β, IL-2, iNOS, COX-2, NF-κB, and MPO, and the production of NO, PGE2, and MCP-1. Increases the IL-10 and GSH	Arunsi et al., 2022

Althaea officinalis Marsh mallow Malvaceae	Althaeae radix, folium et flos	Aqueous extract	Rheumatoid Arthritis Anti-arthritic	Scopoletin	Anti-inflammatory	Reduce inflammation Suppress the release of PGE2, TNF-a, IL-1β, IL-6 and the expression of COX-2	Farzaei et al., 2016 Saleem et al., 2019
Althaea rosea Hollyhock Malvaceae	Malvae arboreae flos sine calicibus	Ethanolic extract	Rheumatoid Arthritis	Oil	Anti-inflammatory	Decrease permeability of abdominal capillaries, and reduce paw edema by downregulating the release of PGE from inflammatory tissue	Farzaei et al., 2016
Arctium lappa Garden celery Asteraceae	Bardanae radix Bardanae semen	Infusion Ethanolic extract Butanolic extract	Anti-arthritic Rheumatoid Arthritis	Arctigenin, arctigenin and its glycoside arctin	Anti-arthritic	Up-regulating expression of VEGF and macrophages that unleash inflammatory cytokines and nitric oxide Downregulate interleukins (IL-1β, IL-6, IL-4, IL-5), NO and TNF-α. Suppress the release of iNOS Inhibitor of MAPK	Choudhary et al., 2015, Saleem et al., 2019, Farzaei et al., 2016
Arnica montana Mountain tobacco Asteraceae	Arnicae flos	Extract methanol Extract total	Osteoarthritis, Anti-arthritic	Phenolic and flavonoid compounds	Anti-inflammatory	Decrease in IL-1β, IL-6, IL-12, NO, TNF-α, anti-collagen II antibodies Increase of antioxidants.	Dragoș et al., 2016, Kang et al., 2019 Zang, 2020 Sharma, 2016

Artemisia absinthium Worm-wood Asteraceae	Absinthii herba	Extract	Rheumatoid Arthritis	Scoparone, scopoletin, scopolin, esculetin	Anti-inflammatory	Suppress the release of NO and PGE-2 (proinflammatory compound) Downregulate COX-2, TNF-α, IL-1β, IL-6, and IL-8	Saleem et al., 2019
Betula sp. Birch Betulaceae	Betulae cortex	Extract	Osteoarthritis	Betulin	Anti-inflammatory	Inhibits gene expression of MMP-13, MMP-1, MMP-3 ADAMTS-4, and ADAMTS-5 induced by IL-1β Up-regulate type II collagen's gene expression. Inhibits the secretion of MMP-3 and its proteolytic activity	Kang et al., 2019 Ra et al., 2017
Borago officinalis Starflower Boraginaceae	Boraginis semen	Oil	Rheumatoid Arthritis	Gamma-linoleic acid	Anti-inflammatory	Significant increase in PGE level due to the reduction in cAMP. Suppress the NF-kB, ERK1/2, and JNK1 pathways and TNF-α.	Singh et al., 2020 Ghasemian et al., 2016
Bryonia alba White bryony Cucurbitaceae	Bryoniae radix		Rheumatoid Arthritis	Cucurbitacin glucoside	Anti-inflammatory	Suppress the mediators of inflammation LT-B4 and 5-HETE and adjust corticosteroid secretion	Gautam et al., 2020
Caltha palustris Marsh-marigold Ranunlaceae	Calthae palustridis herba	Extract-methanol Extract	Anti-arthritic	Polysaccharide	Anti-arthritic	Decrease T-regulatory cells ($CD4^+CD25^+FOXP3^-$) Increase NO	Choudhary et al., 2015 Suszko & Obmińska-Mrukowicz, 2017

						synthesis Downregulate IL-1β	
Cannabis sativus Hemp Urticaceae	Cannabis herba et semen	Extract	Anti-arthritic	Cannabin, cannabion, cannabene, cannabinone, and other terpenes	Anti-inflammatory	Inhibits inflammatory mediators Suppression of TNF-α Immunosuppression of a T-helper 1 effector cells response.	Yashika Gandhi et al., 2022
Capsicum annuum Red pepper Solanaceae	Capsici fructus et semen	tincture	Anti-arthritic	Capsaicin	Anti-arthritic	Suppress collagenase, expression from synoviocytes, and the synthesis of PGE2.	Arunsi et al., 2022
Chelidonium majus Great celandine Papaveraceae	Chelidonii herba	Extract methanol	Rheumatoid Arthritis	Chelidonine	Anti-inflammatory	Lower the CD4+ T cells and enhance CD8+ T cells inducing an immunosuppressive response.	Choudhary et al., 2015
Citrullus colocynthis Colocynth Cucurbitaceae	Colocynthidis fructus	Powder (aqueous extract)	Rheumatoid Arthritis	Quercetin	Anti-inflammatory Anti-arthritic	Downregulate IL-6, IL-1β and COX-2 expression; Increases IL-4	Gautam et al., 2020
Clematis vitalba Old man's beard Ranunculaceae	Clematidis herba	Hydroalcoholic extract	Rheumatoid Arthritis	Vitalboside	Anti-inflammatory	Reduce edema and arthritis.	Farzaei et al., 2016
Colchicum autumnale Autumn crocus Colchicaceae	Colchici bulbus et semen	Extract	Rheumatoid Arthritis	Colchicine	Anti-arthritic Anti-inflammatory	Suppress the production of TNF-α, IL-6, and IL-1β and the expression of TNF-R1 in the synovium	Saleem et al., 2019 Choudhary et al., 2015

Coriandrum sativum Coriander Apiaceae	Coriandri fructus	Oil hydroalcoholic extract from seed, stem, and leaves	Anti-arthritic	γ-Linolenic acid Cineole	Anti-inflammatory Anti-arthritic	Downregulate IL-6, IL-1B and TNF-α, TNF-R1	Yashika Gandhi et al., 2022 Singh et al., 2020 Gautam et al. 2020
Crocus sativus Saffron Iridaceae	Croci stigma	Tincture, infusion Extract	anti-arthritic Osteoarthritis	Crocin, Crocetin, Safranal	Anti-inflammatory, Anti-arthritic	Suppress the NF-κB signaling pathway in joint chondrocytes blocking the expression of MMP-13, MMP-3, and MMP-11 Reduce degeneration of cartilage.	Choudhary et al., 2015 Kang et al., 2019 Tsiogkas et al., 2021
Cymbopogon citratus Citronella Poaceae	Cymbopogonis citrati herba	Oil	Rheumatoid Arthritis	geraniol, neral, limonene, citral	Anti-arthritic	Activate PPARα and γ; Inhibition of COX-2, NO production, Suppress expression of iNOS, DNA-binding activity, and nuclear translocation of NF-κB and IκB kinase phosphorylation	Arunsi et al., 2022
Cuscuta campestris Field dodder Convolvulaceae	Cuscutae semen	Extract metanolic	Rheumatoid Arthritis	Quercetin	Anti-inflammatory	Reducing NO	Farzaei et al., 2016
Cuscuta epithymum Dodder Convolvulaceae	Cuscutae semen	Extract metanolic	Rheumatoid Arthritis	Quercetin	Anti-inflammatory	Mediate the suppression of NF-κB expression through the downregulation of cytokines, COX-2 and TNF-α.	Saleem et al., 2019

Elaeagnus angustifolia Oleaster Elaeagnaceae	Elaeagni fructus	aqueous and ethanol extracts	Osteoarthritis	Kaemferol	Analgesic Anti-inflammatory	Inhibits COX-1 and COX-2 Downregulate TNF-α and IL-6, mediators (NO and PGE2) Signaling kinases (Src, Syk, and IRAK4), and release of ROS.	Panahi et al., 2016
Ephedra sinica, *Ephedra spp.* Chinese ephedra Ephedraceae	Ephedrae herba	Aqueous extract	Rheumatoid Arthritis Anti-arthritic	Polysaccharides, Ephedrine alkaloids	Anti-inflammatory, immunosuppressive, analgesic Anti-arthritic	Suppress the TLR4 signaling pathway and reduce NF-κB, downregulating the release of inflammatory factors and cytokines. Regulate the expressions of TNF-α and IL-6 genes.	Xia et al., 2020 Choudhary et al., 2015
Equisetum arvense Horsetail Equisetaceae	Equiseti herba	Extract	Osteoarthritis Rheumatoid Arthritis	Kynurenic acid	Anti-inflammatory, anti-oxidative, and pain-relieving properties	Reduce synoviocyte proliferation Downregulate the level of TNF-α and IL-10.	Dragoş et al., 2017 Jiang, 2014
Fraxinus excelsior European ash Oleaceae	Fraxini folium	Decoction of dried leaves	Anti-arthritic	flavonoids: rutoside, quercitroside, phenolic acids: caffeic acid, coumarin derivatives: esculoside, fraxoside, secoiridoids: excelsioside, oleuropein, ligstroside, mucilage.	Anti-inflammatory	Inhibition of xanthine oxidase	Villeneuve, 2017

Glycyrrhiza glabra Liquorice Fabaceae	Liquiritiae radix	Extract methanol Extract	OsteoArthritis, Anti-arthritic	Flavonoids Licochalcone A Glycyrrhetinic acid Liquiritin, glycyrol, glycyrrhizin Prunetin	Anti-inflammatory	Suppresses bone and cartilage erosion. Suppresses the expression of IL-1β, IL-18 and NLRP3 Inhibits COX-2, production of MMP-3 stimulated by IL-1β	Choudhary et al., 2015 Yashika Gandhi et al., 2022 Xia et al., 2020 Singh et al., 2020 Kang et al., 2019
Hedera helix English ivy Araliaceae	Hederae helicis folium	Extract ethanol	Rheumatoid Arthritis/injection	Saponin	Anti-inflammatory	Reduction in arthritic symptoms	Choudhary et al., 2015 Rai, 2013
Humulus lupulus Hop Cannabaceae	Lupuli strobuli, Lupuli glandulae	Infusion		humulone	Anti-arthritic	Suppress COX-2 gene transcription Inhibition of PGE_2 production	Choudhary et al., 2015 Hougee et al., 2006
Inula helenium Elecampane Asteraceae	Inulae rhizoma et radix	Extract	Rheumatoid Arthritis	Dihydroflavonols Sesquiterpene lactones, mainly alantolactone, isoalantolactone	Anti-inflammatory	Inhibition of TNF-α, MCP1, MCP2, MMP3, IL-1, IL-6	Midhun Kumar et al., 2020 Singh et al., 2020
Linum usitatissimum Flaxseed Linaceae	Lini semen	Petroleum ether	Anti-arthritic	alpha linolenic acid	Anti-arthritic	Inhibition of arachidonate metabolism through suppressing the production of n-6 eicosanoids PGE2, LTB4, and diminishing vascular permeability	Choudhary et al., 2015 Kaithwas et al., 2010
Lonicera japonica Japanese honeysuckle Caprifoliaceae	Lonicerae folium	Extract methanol	Anti-arthritic	Luteolina	Anti-inflammatory	Suppress T-cell proliferation	Choudhary et al., 2015

Matricaria chamomilla Camomile Asteraceae	Chamomillae flos	Oil Tea	Osteoarthritis Rheumatoid Arthritis	apigenin, quercetin, patuletin,luteolin and glucozide	Anti-arthritic Anti-inflammatory	Reduction in need for acetaminophen. Reducing cytokines and PGE2	Lindler et al., 2020
Nigella sativa Black cumin Ranunculaceae	Nigellae semen	Aqueous extract Extract	Anti-arthritic, Rheumatoid Arthritis	Thymoquinone	Anti-inflammatory Anti-arthritic	Reduce TLR2, TLR4, TNF-α, NFκB, the levels of COX-2, IL-1 β, IL-6, IL-8, IL-12, and IL-16.	Arjumand et al., 2019 Yashika Gandhi et al., 2022 Saleem et al., 2019
Ocimum basilicum Basil Lamiaceae	Basilici herba	Extract	Rheumatoid Arthritis	Rutin (Vitamin P)	Anti-arthritic	Inhibition in oxidative stress markers of NO, T cells proliferation and $NADP^+$ oxidase. Reduce edema, cartilage and bone erosion. Up-regulation of SOD, GPx, and GSH, downregulation of MDA levels. Inhibition of TNF-α, IL-1β, and NF-κB.	Arunsi et al., 2022
Oenothera biennis Evening primrose Onagraceae	Oenotherae semen	Extract	Rheumatoid Arthritis	GLA-gamma-linoleic acid	Anti-inflammatory	Modulation of nitric oxide (NO), TNF-α, IL-1β and TXB2 resulting in suppressing of COX-2	Singh et al., 2020

Osmunda regalis Royal fern Osmundaceae	Osmundae herba	Extract	-	-	Anti-arthritic	-	Choudhary et al., 2015
Peganum harmala Wild rue Zygophyllaceae	Pegani harmalae folium seeds	Decoction Peganum oil	Rheumatoid Arthritis	Alkaloids	Anti-arthritic Analgesic	Reducing pain and difficulty in function	Choudhary et al., 2015 Abolhassanzadeh et al., 2015
Phellodendron amurense Amur cork tree Rutaceae	bark	Extract ethanol	Osteoarthritis	Palmatine, protoberberin	Anti-inflammatory Analgesic	Suppress IL-1β-stimulated type II collagen degradation and release of proteoglycans leading to cartilage protection.	Kang et al., 2019
Physalis alkekengi Strawberry tomato Solanaceae	*Alkekengi* fructus	Extract Aqueous	Anti-arthritic	Polyphenols Physalins, sophysalin B, and aromaphysalin B	Anti-arthritic, anti-inflammatory	Inhibits protein denaturation Reduce NO production; Suppress TNF-α, IL-6 and IL-12	Choudhary et al., 2015 Yang Y. et al., 2022
Pinus cembra Swiss stone pine Pinaceae	Pinis cembra propolis	Extract	Osteoarthritis	Pinocembrin (flavonoid)	Protective effect	Inhibits MMP-1, MMP-13, and MMP-3 expression by modulating the NF-κB signaling pathway	Kang et al., 2019 Zhang et al., 2015
Plantago major Broadleaf plantain Plantaginaceae	Plantaginis folium	Extract	Anti-arthritic	n-hexane-insoluble fraction of dichloromethane extracts	Anti-arthritic	Decrease expression of TNF-α and IL-6.	Choudhary et al., 2015 Triastuti et al., 2021
Populus nigra L. Black poplar Salicaceae	Populi gemma	Extract	Anti-arthritic	Flavonoids	Anti-inflammatory	Adjust the production of TNF-α, IL-1β, IL-6, IL-10	Oroian, 2011 Kis et al., 2020 Oroian et al., 2019
Portulaca oleracea Common purslane, Pursley Portulacaceae	Portulace folium	Petroleum ether extract Juice, poultice	Anti-arthritic	alkaloids, tannins, flavonoids, saponins, and triterpenoids	Anti-inflammatory Anti-arthritic	Downregulate IL-1, IL-6, and TNF-α	Jaya Sankar Reddy et al., 2014 Choudhary et al., 2015 Nadipelly et al., 2012

							Allahmoradi et al., 2018
Ranunculus sceleratus Celery-leaved buttercup Ranunculaceae	Ranunculi sceleratus herba	Extract	Rheumatoid Arthritis	myristic acid, Phytol, B-sitosterol, Stigmasterol, Ranunculin	Anti-inflammatory	Suppress production of eicosanoid, PLA2, and 12-LOX pathway	Gautam et al., 2020
Reynoutria japonica Japanese knotweed Polygonaceae	Reynoutriae radix	Extract	Osteoarthritis	Resveratrol	Anti-inflammatory	Block the NF-κB signaling pathway leading to suppression of iNOS, COX-2, TNF-α, and IL-1β expression; Inhibits the MMP-3 gene expression and secretion	Kang et al., 2019 Cucu et al., 2021 Dursus, 2018 Nawrot-Hadzik et al., 2021
Rheum palmatum Chinese rhubarb Polygonaceae	Rhei rhizoma	Extract	Rheumatoid Arthritis	Emodin	Anti-inflammatory,	Regulate TNF-α, iNOS, and IL-10, IL-6, IL-8, PGE2, MMP-1, COX-2, VEGF as well as NF-jB	Farzaei et al., 2016 Saleem et al., 2019
Ribes nigrum Blackcurrant Grossulariaceae	Ribes nigri muguri	Extract	Rheumatoid Arthritis	α-linolenic acid Flavonoids Proanthocyanidins Phenolic acids Vitamin C	Anti-inflammatory, analgesic; anti-arthritic	inhibiting the expression of IL-6 and TNF-α iNOS, and IL-10 as well as NF-jBp65	Musco et al., 2019 Villeneuve, 2017
Ricinus communis Castor Oil Euphorbiaceae	Ricini semen	Oil, poultice	Rheumatoid Arthritis	Polyphenols and flavonoids	Anti-inflammatory	Suppress IL-1β, IL-6, IL-17a, TNF-α, and RANKL Modulation of IL-4, INF-gamma, and OPG expression	Choudhary et al., 2015

Ruta graveolens Common rue Rutaceae	Rutae herba	Extract	Anti-arthritic	Polyphenols	Anti-inflammatory	Down-regulate TBARS, COX-2, 5-LOX and MPO level Reduce NO, TNF-α, IL-1β, and IL-6	Choudhary et al., 2015 Sahu et al., 2015
Salix alba White willow Salicaceae	Salicis cortex	Decoction Aqueous Extract	Osteoarthritis and Rheumatoid Arthritis	salicin, polyphenols, flavonoids	Anti-arthritic anti-inflammatory	Inhibit translocation of the transcription factor NF-κB Regulate COX-1, COX-2, 5-lipoxygenase (5-LOX), TNF-α, IL-1, IL-6, and NO	Dragoș et al., 2017 Bonatera et al., 2010 Henrotin, Y., & Mobasheri (2018)
Saussurea lappa Costus Asteraceae	Costus radix	Extract	Anti-arthritic	Cynaropicrin Dehydrocostus	Anti-inflammatory anti-arthritic	Inhibits production of TNF- α Supresses the production of LPS-induced NO	Yashika Gandhi et al., 2022
Sesamum indicum Sesame Pedaliaceae	Sesami oleum	Oil	OsteoArthritis and Rheumatoid Arthritis	sesamin, sesamol,sesamolin	Anti-arthritic, anti-inflammatory	Regulate TNF- α, IL-1β, IL-6, COX-2, MMP-13, MMP-3, MMP-9 gene expression	Dragoș et al., 2017 Askari, et al., 2019
Solanum melongena Eggplant Solanaceae	Solani melongena fructus	Extract	Rheumatoid Arthritis	Apigenin	Anti-inflammatory	Decreases the level of TNF-α, IL-1β, and IL-6 Inhibits CXCR4 gene expression.	Arunsi et al., 2022
Solanum nigrum Black nightshade Solanaceae	Solani nigri folium	poultice		Solanine A	Anti-arthritic	Suppresses TNF-α, IL-1β, and IL-6 and NF-κB	Choudhary et al., 2015 Zhao et al., 2018
Symphytum officinale Comfrey Boraginaceae	Symphyti radix	Extract hydroalcoholic extract	OsteoArthritis and Rheumatoid Arthritis	rosmarinic acids, glycopeptides, amino acids	Anti-inflammatory, analgesic	Regulate pain and articular mobility Suppresses activation of NF-κB Regulate IL-1, and COX-2	Dragoș et al., 2017 Kang et al., 2019 Seigner et al., 2019

Thymus vulgaris Garden thyme Lamiaceae	Thymi herba	Oil	Anti-arthritic	Carvacrol (monoterpene phenol)	Anti-inflammatory	reduce TNF-α IL- 8 and IL−6 inhibit MMP- 1, MMP- 3, and MMP- 13suppresses activation of NF- κB	El-Sheikh et al., 2019
Tribulus terrestris Land-Caltrops Zygophyllaceae	Tribuli terrestris fructus	methanolic extract	Anti-arthritic	N -trans- p -caffeoy l tyramine Tribulusamide D	Anti-inflammatory	Modulate COX-2, TNF-α, IL-6, and NO synthase 2 Inhibits MMP-2 and MMP-9 expression	Choudhary et al., 2015 Yashika Gandhi et al., 2022 Jaya Sankar Reddy et al., 2014 Park et al., 2017
Urtica dioica Common nettle Urticaceae	Urticae folium	Extract hydroalcoholic extract	Osteoarthritis and Rheumatoid Arthritis	Carvacrol, carvone, chlorogenic acid, phaselic acid, rutin Neophytadiene, Phtaleic acid, Dibutyl phthalate, Bis (2-ethyl hexyls') maleate, 1,2-benzenocli carboxylic acid	Anti-inflammatory	Inhibits NF-κB and AP-1 activation Block cytokines expression and eicosanoids formation Suppresses expression of (MMPs)-9 and 3	Gautam et al., 2020 Anvari et al., 2022 Goswami et al., 2022 Villeneuve, 2017
Vitis sp. Grapevine Vitaceae	Vitis viniferae semen	Extract	Osteoarthritis	Proanthocyanidins	Antioxidant Anti-inflammatory	Inhibits NO, PGE2, TNF-α IL-1β, and IL-17	Anvari et al., 2022
Xanthium strumarium Common cocklebur Asteraceae	Xanthii herba	Ethanol extact	anti-arthritic	alkaloids	Anti-inflammatory	Downregulate TNF-α, IL-1β; COX-2 and 5-LOX. Increase IL-10.	Choudhary et al., 2015 Lin et al., 2014

Abbreviations: IL-1β: interleukin 1 beta; IL-4: interleukin 4; IL-5: interleukin 5; TNF-α: tumor necrosis factor-alpha; TNF-R1: Tumor necrosis factor receptor 1; E-NTPDase: ecto-nucleoside triphosphate diphosphohydrolase; IFN-gamma: Interferon-gamma; CRP: c-reactive protein; COX-2: cyclooxygenase-2; PGE2: prostaglandin E2; VEGF: vascular endothelial growth factor; iNOS: inducible nitric oxide synthase; MAPK: mitogen-activated protein kinase; NO: nitric oxide; cAMP: cyclic adenosine monophosphate; NF-κB: Nuclear factor kappa B; LTB4: Leukotriene B4; 5-HETE: 5-Hydroxyeicosatetraenoic acid; IkB: inhibitor of nuclear factor; Src: Human Recombinant Protein, Syk: Tyrosine-protein kinase); IRAK-4: interleukin-1 receptor-associated kinase 4; ROS: reactive oxygen species; NLRP3: NLR family pyrin domain containing 3; MCP1, 2: Monocyte chemoattractant protein-1, 2; NADP+: Nicotinamide adenine dinucleotide phosphate; TXB2: thromboxane B2; PLA2: phospholipase A_2; 12-LOX: 12-Lipoxygenase; NF-jB: Stimulus-induced nuclear factor-jB; RANKL: Receptor activator of nuclear factor kappa-B ligand; OPG: Osteoprotegerin; TBARS: Thiobarbituric acid reactive substances; AP-1: activator protein 1; MMP: Matrix metallopeptidase.

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