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VALORIZATION OF THE MEDICINAL PLANT *VACHELLIA HORRIDA (L.)* KYAL. & BOATWR. THROUGH CHEMICAL AND BIOLOGICAL EVALUATION OF ITS EXTRACTS

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Abstract: This study aims to enhance the value of the medicinal plant *Acacia horrida* (synonym of *Vachellia horrida*) by evaluating its chemical and biological properties, specifically focusing on its antioxidant and enzymatic activities. Three extraction methods, namely ultrasonication, maceration, and microwave-assisted extraction, were employed, utilizing various solvent systems to extract bioactive molecules, quantify total polyphenols and flavonoids, and measure antioxidant, anti-inflammatory, antidiabetic, and antimicrobial activities. The antioxidant activity was assessed using several tests, including DPPH and ABTS radical scavenging assays, as well as the CUPRAC and FRAP methods for measuring reducing power. The radical scavenging tests proved to be the most effective. Phytochemical screening of the extracts revealed an abundance of secondary metabolites, particularly total polyphenols and flavonoids, in the ethyl acetate (AE) extract, which likely accounts for its potent antioxidant activity. The study confirms the efficacy and high yield of the ultrasonication method compared to maceration and microwave-assisted extraction, as mentioned earlier. On the other hand, the extracts from this plant exhibited no significant antidiabetic or anti-inflammatory activities. However, they did demonstrate antibacterial activity, with the dichloromethane (DC) and butanolic (BU) extracts displaying notable effects.

Keywords: *Acacia horrida*, antioxidant activity, biological activities, total polyphenols, flavonoids, extraction

1. Introduction

Woody plants, especially trees, captivate our interest with their stems, trunks, canopies, and branches, collectively encompassing their aerial parts. Their majestic beauty and ornamental richness often leave a profound impression on humans, drawing immediate attention to their overall appearance (Zhao et al., 2015; Heywood, 1996; Spichiger et al., 2002). Shrubs, being woody plants with entirely lignified stems branching from the base, manifest in various forms, including single-stemmed, multi-stemmed, or bushy types (Belot, 2004). When a woody plant fails to reach a height of at least 3 meters due to multiple stems or small size, it is classified as a shrub (El Amin, 1990). Epidemiological, *in*

vitro, *in vivo*, and clinical studies have demonstrated that a plant-rich diet can reduce the risk of certain degenerative diseases, such as diabetes, obesity, cardiovascular complications, and cancer (Bruneton, 1999; Gaston, 2016; Afonso et al., 2007). For instance, research indicates that approximately 20 to 50% of all cancer cases can be prevented through plant-based diets (Glade, 1999).

One such widespread shrub across the globe is *Acacia* genus, comprising approximately 1200 species within the Fabaceae family, belonging to the tribe Acacieae. *Acacia* species hold significance in nutrition, ethnopharmacology, and offer potential therapeutic uses (Halliwell, 1999; Subhan et al., 2018; Habellah et al., 2016). Notably, 152 chemical constituents have been identified from Acacia species, primarily concentrated in the leaves, pods, and stem bark, with flavonoids, terpenoids, and phenolic acids being the major isolated compounds. These bioactive compounds exhibit diverse pharmacological activities, such as antibacterial, antifungal, antioxidant, and anticancer effects (Amoussa et al., 2020; Ghedira, 2005).

Studies have revealed that *Acacia* plants foliage presents highly nutritious fodder, particularly rich in proteins. In Algeria, five species belonging to the Fabaceae family, *A. nilotica* (synonym of *Vachellia nilotica*), *A. horrida* (synonym of *Vachellia horrida*), *A. saligna*, *Faidherbia albida*, and *Albizia julibrissin* collected from arid and semi-arid zones demonstrated high protein contents (157-252 g/kg dry matter), with *A. horrida* exhibiting the highest content (551 g/kg dry matter) (Kadi and Zirmi-Zembri, 2016). Consequently, the leaves of these forage shrubs present a promising alternative feed source for ruminant animals, considering the forage deficits in Algeria and the rising costs of raw materials used in concentrated feeds (Mebirouk-Boudechiche et al., 2014). Furthermore, the *Acacia* genus is renowned for its abundance of secondary metabolites. Studies on ten *Acacia* species reported the presence of seventy-six (1-76) molecules, including triterpenes, saponins, flavonoids, polyphenols, tannins, among others (Hussain, 2019).

The objective of this study is to valorize the species *A. horrida*, for which limited data are available concerning its biological properties. Indeed, the research aims to extract bioactive molecules using innovative methods, such as the ultrasonication and microwave-assisted extraction techniques, rather than the conventional approach. Additionally, this study intends to develop biological activity tests, including antioxidant activities, anti-inflammatory activity, antibacterial activity and antidiabetic activity. By pursuing these objectives, we aim to shed light on the potential medicinal properties of *A. horrida* and contribute valuable insights to its biological profile.

2. Materials and methods

The study was conducted at the Biotechnology Research Center (CRBT), in Constantine province, Algeria

2.1. Plant material preparation

In this study, the aerial part, particularly the leaves, of *A. horrida* was used. The plant was collected on March 2023 from Constantine Province of Algeria. The species identification was performed at the Laboratory of Molecule Synthesis and Characterization, CRBT Constantine. The aerial part was cleaned with tap water to remove dust and other contaminants then it was oven dried at 37°C for twenty-four hours. After drying, it was ground into a fine powder using an electric grinder (E8130 Waring blender) **(Fig. 1.)** and stored in small glass jars in dark at 4°C until further use. The obtained powder was subjected to different extraction methods.

Fig. 1. Preparation of *Acacia horrida*

Fig. 2. Different Erlenmeyer flasks prepared for each extraction method

2.2. Extraction Methods

Different extraction methods were used for the extraction of plant essences, namely: solid-liquid extraction by maceration, extraction by ultrasonication and microwave-assisted extraction. For each method, a mass of approximately 50g was immersed in a hydoalcoholic solution (ethanol-water), and then placed in Erlenmeyer flasks **(Fig. 2.)**.

2.2.1. Solid-liquid extraction by maceration

Maceration is a process that involves letting a solid substance soak in a cold liquid to extract soluble compounds or to absorb the liquid to obtain its fragrance or flavor, preserve it, or allow it to decompose. Ethanol is often used to extract phenolic compounds.

A mass of 50g ground plant material were subjected to maceration in 300 ml of a hydroalcoholic solution (ethanol + water). The mixture was mechanically stirred for 24 hours.

After vacuum filtration, two additional extractions on the residue were performed. Subsequently, the ethanolic extract was evaporated under vacuum using a rotary evaporator (BUCHI) at a temperature of 38°C. The mixture was then dissolved in an ultrasonic bath (Elmasonic S70 H) , and the crude extract was collected in a petri dish and dried in an oven (ETUVE MEMMERT UF160 - 161L) at 37°C until all the methanol was evaporated.

2.2.2. Extraction by ultrasonication

Ultrasonic extraction is a rapid, cost-effective, simple, and efficient alternative method that offers numerous advantages in terms of yield, selectivity, time, and energy.

A quantity of 50g of ground plant material was placed in an Erlenmeyer flask and

macerated in 200 ml of a hydroalcoholic solution.

The mixture was allowed to sit for 24 hours. The Erlenmeyer flask was then placed in an ultrasonic extractor and subjected to extraction for 30 minutes. The extract was subsequently evaporated under vacuum at 38°C until the solvent was completely evaporated. Finally, the extract was recovered and dried at 37°C in an oven.

2.2.3. Microwave-assisted extraction

In this method, a microwave oven was used, and the following procedure was adopted: In a beaker, an equal-volume hydroalcoholic mixture (320 ml ethanol + 80 ml H_2O) was added to 70 g of the sample. The experimental setup was then placed in a microwave oven (Samsung ME73A) and subjected to microwave irradiation for 90 seconds at a pressure of 600 mbar.The mixture was recovered, followed by vacuum filtration. Subsequently, the extract underwent evaporation under vacuum at 38°C until the solvent was completely evaporated. Finally, the extract was collected and dried at 37°C in an oven.

2.3. Estimation of total phenols and flavonoids content

2.3.1. Estimation of total phenols content (TPC)

The determination of polyphenols content was carried out according to the method described by (Muller et al., 2012), on a96-well microplate, a volume of 20 µl of extract (1 mg of extract dissolved in 1 ml of methanol) is added to 100 µl of diluted FCR. Then, 75 µl of sodium carbonate (7.5%) is added, and the mixture is kept in the dark for 2 hours at room temperature. The absorbance is measured at 765 nm. A blank is prepared in the same manner by replacing the extract with the solvent used (Methanol). Gallic acid is used as a positive control, and a calibration curve is established based on it.

2.3.2. Estimation of total flavonoid content (TFC)

The test was conducted by using a 96-well microplate according to the method described by (Khlifi et al., 2013), a volume of 1 ml of each extract was mixed with an equal volume of aluminum trichloride (AlCl3) in methanol (2%). (The volumes were scaled down by a factor of 10.) The mixture was kept in the dark for 15 minutes at room temperature. The absorbance was read at 415 nm. A blank was prepared in the same manner by replacing the extract with the solvent used (Methanol). Quercetin was used as a positive control, and a calibration curve was established based on it.

2.4. *In vitro* biological activities assessment of *Acacia horrida* extracts

Evaluation of biological activities was conducted using a PerkinElmer 96-well microplate reader with EnSpire software.

2.4.1. Antioxidant activity

The antioxidant activity of extracts of the species *Acacia horrida* was carried out using various methods: DPPH free radical scavenging, ABTS radical scavenging, Ferric Reducing Antioxidant Power (FRAP), and copper-neocuproine complex (CUPRAC).

2.4.1.1. Free radical scavenging test of DPPH

The test was carried out according to the method described by (Bloi, 1958). On 96-well microplate, a solution of 160 µl of DPPH was mixed with 40 µl of the extract at different concentrations. The mixture was kept at room temperature for 20 minutes, and the absorbance was measured at 517 nm. Ascorbic acid and trolox were used as standards.

2.4.1.2. Test of ABTS radical scavenging

The test was carried out according to the method described by (Re et al., 1999), a concentrations, the absorbance of the reaction medium was measured at 734 nm after 10 minutes of incubation. Ascorbic acid and trolox were used as standards, and their absorbance was measured under the same conditions as the samples.

2.4.1.3. Reducing power activity (FRAP)

The reducing power of iron (Fe^{3+}) was determined using the method described by (Oyaizu, 1986). This method is based on the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) by antioxidants, resulting in a blue color formation. In this assay, a volume of 10 µl of the extract at different concentrations was mixed with 40 µl of a phosphate buffer solution (pH 6.6) and 50 µl of a potassium ferricyanide solution (1%). The mixture was incubated at 50°C for 20 minutes. After incubation, 50 µl of 10% trichloroacetic acid (TCA) was added to stop the reaction. Finally, 40 µl of distilled water and 10 µl of a ferric chloride solution (0.1%) were added.

The absorbance of the reaction medium was measured at 700 nm. Ascorbic acid and trolox were used as standards, and their absorbance was measured under the same conditions as the samples.

2.4.1.4. Copper-neocuproine reducing power test (CUPRAC)

The CUPRAC method is based on the reduction of the Copper-Neocuproine complex in the presence of an antioxidant. It is determined according to the method of (Apak et al., 2014).In a 96-well microplate, 40 µl of the sample was added to a mixture containing 60 µl of ammonium acetate buffer, 50 µl of Neocuproine, and 50 µl of copper chloride ($CuCl_2$). After one hour of incubation, the absorbance was measured at 450 nm. Ascorbic acid and trolox were used as standards, and their absorbance was measured under the same conditions as the samples.

volume of 160 µl of ABTS was added to 40 µl of the extract diluted in methanol at various

2.5. Antidiabetic activity (alpha-amylase inhibition)

The inhibitory activity of alpha-amylase was determined using the method of (Zengin et al., 2014) with some modifications. In a 96-well microplate, a volume of 25 µl of the extract at different concentrations was mixed with 50 µl of alpha-amylase solution (1U/ml), then incubated for 10 minutes at 37°C. Subsequently, 50 µl of starch solution (0.1%) was added to the mixture, which was incubated again for an additional 10 minutes at 37°C.

After incubation, 25 µl of hypochlorous acid (1M) and 100 µl of potassium iodide solution were added. The absorbance was measured at 630 nm. Acarbose was used as a standard reference for comparison.

2.6. Anti-inflammatory activity

The anti-inflammatory activity was determined by thermal denaturation of BSA (Kandikattu et al., 2013) with some modifications. A volume of 100 µl of each extract was mixed with 100 µl of Bovine Serum Albumin solution (0.2%) prepared in Tris-HCl buffer (50 mM, pH 6.6). The mixture was allowed to stand for 15 minutes at 37 °C then heated in a water bath at 72 °C for 5 minutes. Absorbance was recorded at 660 nm after cooling to room temperature. Sodium diclofenac was used as a standard.

2.7. Antibacterial activity

The antibacterial activity was determined using the disc diffusion method, known as the aromatogram technique. This involves placing discs soaked in the extract on a solid medium

inoculated with bacterial strains through swabbing, to assess the sensitivity or resistance of the strains to these compounds. The five tested extracts were: methanolic extract, dichloromethane, ethyl acetate, butanolic, and sonication-derived extract.

2.7.1. Pathogenic microbial strains

The antibacterial activity of *Acacia horrida* extracts was assessed against: *Escherichia coli ATCC* 25922 and *Staphylococcus aureus ATCC* 25923.

2.7.2. Application of discs and incubation

Discs of 6 mm diameter made from Wattman paper soaked with the extracts were placed on the surface of Mueller Hinton agar inoculated with the tested bacteria using sterile forceps. Similarly, discs soaked with methanol-water solvent (negative control) and discs of the antibiotic cefotaxime 30 µg (positive control) were placed. Petri dishes are left for 1 hour at room temperature for pre-diffusion, then incubated at 37°C for 24 hours.

2.7.3. Reading and interpretation

Results are interpreted by measuring the zones of inhibition after 24 hours of incubation, represented by a clear halo formed around each disc. The results are expressed according to four levels of activity **(Table 1.)**.

2.8. Statistical analyses

The *in vitro* test results were expressed as the mean ± standard deviation of three trials. The IC50 (inhibition concentration at 50%) and A0.50 (concentration indicating 0.50 of absorbance) values were calculated using linear regression. Multiple comparisons and significance rates were determined by the Tukey's honestly significant difference (HSD) test and univariate ANOVA. Differences were considered statistically significant at a threshold of 0.05.

3. Results

3.1. Total phenols and flavonoids content

The values of total phenols and flavonoids content expressed in (µg/mg extract) of the six extracts of *Acacia horrida* plant: EC: classical extract, ES: sonication extract, EM: microwave extract, DC: dichloromethane extract, AE: ethyl acetate extract, BU: butanol extract, are represented in **Table 2**.

The total phenolic compouns content **(Fig. 3.)**, demonstrate that the highest total phenolic content is recorded for the ethyl acetate extract (435.99±0.76 µg/mg), followed by the sonication extract (SE) (240.63±0.57), and microwave extract (ME) (223.96±1.07). The classical extract (CE) displayed a relatively low value of phenolic compounds (129.54±3.8), while the butanolic extract (Bu) showed the lowest content of phenolic compounds.

The results of flavonoid contents for the various extracts illustrated in table 2 show average values. The highest value is recorded for ES (70.68±3.43) and the lowest for EC **(Fig. 4.)**. Both BU and DC extracts do not contain flavonoids.

3.2. Assesment of biological activities

3.2.1. Antioxidant activity

3.2.1.1. DPPH Test

The antioxidant effect of the studied extracts, represented by their IC_{50} values, shows that the EA has a strong antioxidant power with a value of (5.44±1.28 µg/ml), close to that of the tested standard, Trolox. followed by SE (16.9±0.70 µg/ml), EC (25.96±3.57µg/ml), ME (30.57±0.27 µg/ml), and BU (69.48±1.82µg/ml). The DC extract recorded the lowest antioxidant power (>800 µg/ml) **(Fig. 5.)**.

Table 1. Sensitivity of microbial strains based on inhibition zones (Pouce et al., 2003)

Sensitivity	**Inhibition Zone**
Not sensitive or resistant (-)	Diameter < 8 mm
Sensitive (+)	Diameter between 9 and 14 mm
Very sensitive (++)	Diameter between 15 and 19 mm
Extremely sensitive (+++)	Diameter > 20 mm

Table 2. Total polyphenol and flavonoid contents of the 6 extracts of *Acacia horrida*

Extract	**Phenolic compounds content (µg EAG/ mg extract)**	**Flavonoids compounds content (µg EQ/ mg extract)**
Classic Extract (CE)	129.54±3.8	28.86±2.62
Sonication extract (SE)	240.63±0.57	70.68±3.43
Microwave extract (ME)	223.96±1.07	59.39±3.41
Dichloromethane extract (DC)	-	-
Ethyl acetate extract (EA)	435.99±0.76	47.04±3.15
Butanol extract (BU)	58.02±3.41	-

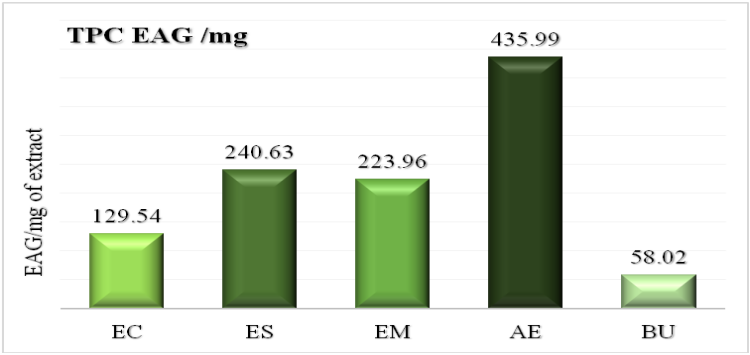


Fig. 3. Total phenolic compound content

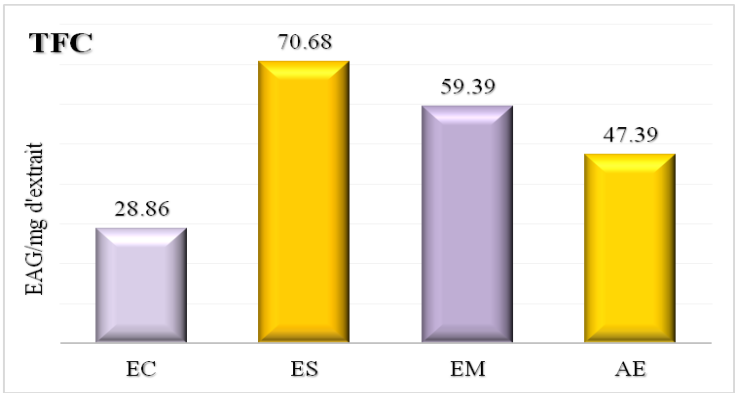


Fig. 4. Total Flavonoid Content

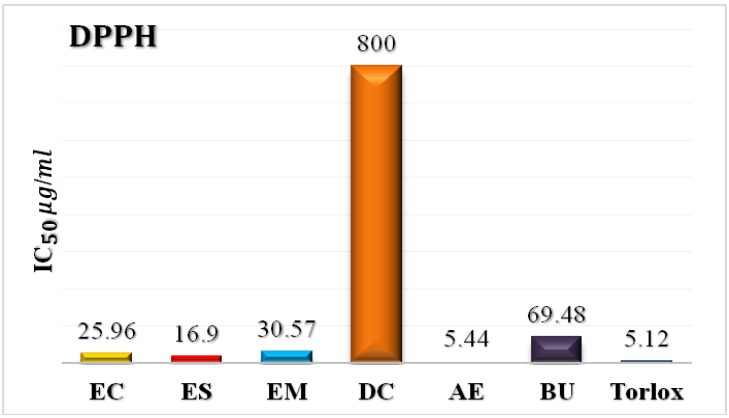


Fig. 5. IC_{50} Values of the Five Extracts of *Acacia horrida* and Trolox for the DPPH Test

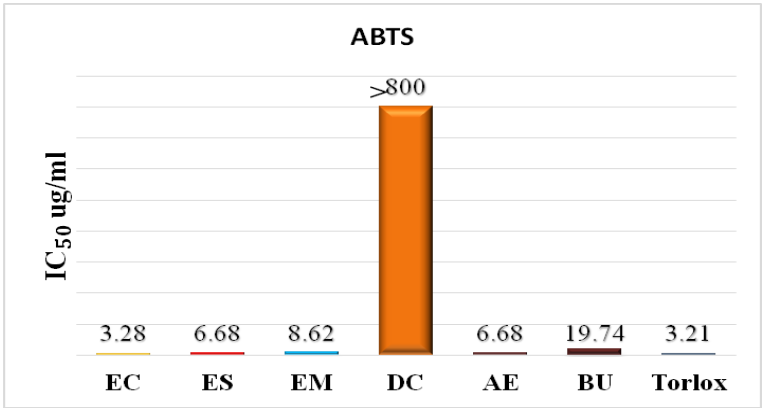


Fig. 6. IC_{50} values of the five extracts of *Acacia horrida* and Trolox for the ABTS test

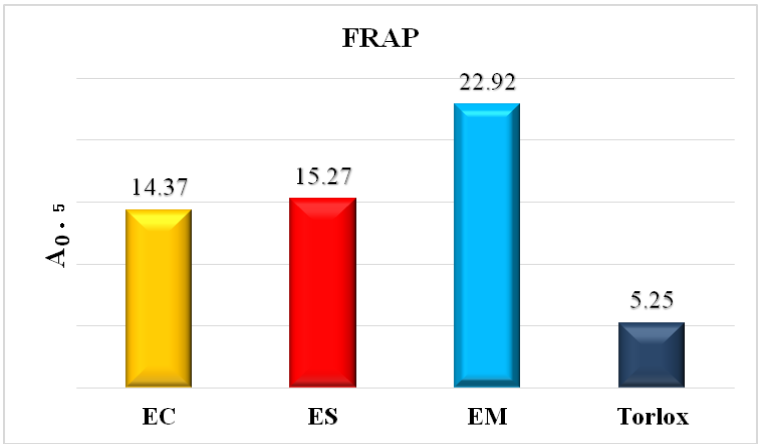


Fig. 7. $A_{0.5}$ values of the extracts compared to the standard for the FRAP test

3.2.1.2. The ABTS radical scavenging test

The results of the antioxidant power by ABTS radical scavenging of the studied extracts, represented by their IC_{50} values **(Fig. 6.)**, revealed that the CE, SE, EA, and ME extracts have a powerful antiradical effect close to that of Trolox.The BU extract shows a relatively weak effect compared to the other extracts, while the DC extract exhibits the lowest activity (> 800 µg/ml).

3.2.1.3. Ferric Reducing Power Test

The results expressed in terms of $A_{0.5}$ (µg/ml) are represented in **Figure 7**.

The three extracts SE,CE,ME show values close to each other but slightly lower compared to Torlox. The extract closest to Torlox is EC.

3.2.1.4. Copper Reduction Antioxidant Capacity (CUPRAC) Test

The $A_{0.5}$ values are of the studied extracts are illustrated in **Figure 8**.

The three tested extracts exhibited weak ferric reducing activities lower than that obtained by Torlox.

3.2.2. Antidiabetic activity

Alpha-amylase is a digestive enzyme that catalyzes the hydrolysis of α-D-glucose polymers at α (1-4) linkages. This enzyme is present in the pancreas and plays an essential role in the digestion of starch present in the food bolus. According to the comparative histogram **(Fig. 9.)**, all tested extracts of *Acacia horrida* show no antidiabetic effect compared to the standard Acarbose.

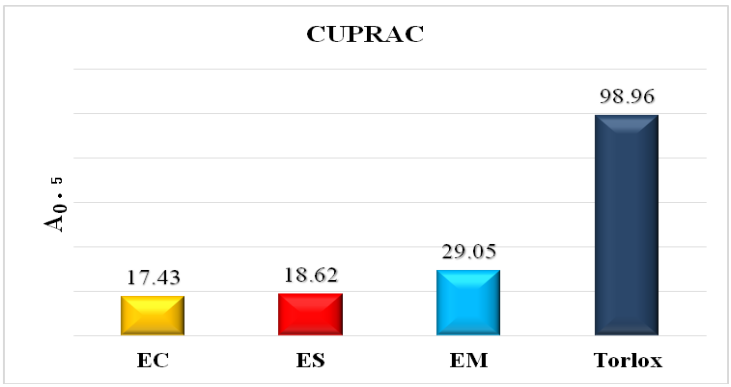


Fig. 8. $A_{0.5}$ values of the extracts compared to the standard for the CUPRAC test

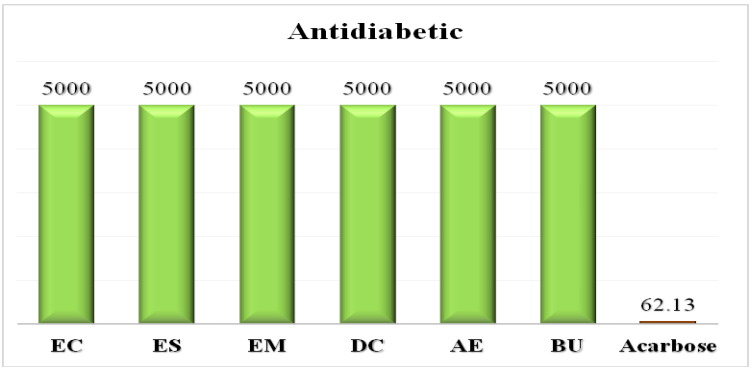


Fig. 9. Comparative histogram of IC_{50} values of the six extracts of *Acacia horrida* with the standard Acarbose

3.2.3. Anti-inflammatory activity

The anti-inflammatory activity was measured relative to the anti-inflammatory standard DICLOFENAC **(Fig. 10)**. According to the results, all extracts of *Acacia horrida* did not exhibit anti-inflammatory activity; the values of the extracts were significantly different from those of the standard Diclofenac.

3.2.4. Antimicrobial activity

Five extracts were tested of their antimicrobial power: CE, DC, EA, BU, SE. Only the extracts DC and BU exhibited clear halos of 9 and 7 mm respectively formed around the discs **(Fig. 11)**.

BU exhibited antimicrobial activity against the bacterium *Staphylococcus aureus ATCC* 25923 and DC showed antimicrobial activity against bacterium *Escherichia coli ATCC* 25922.

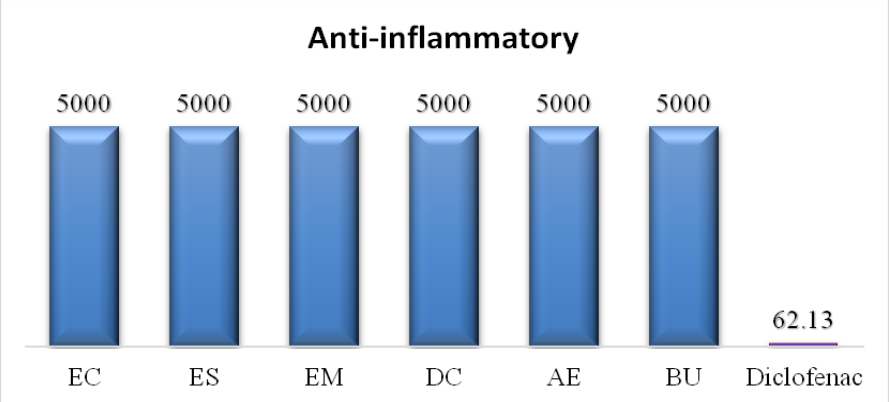


Fig. 10. Comparative histogram of IC50 values of the six extracts of *Acacia horrida* with the standard DICLOFENAC

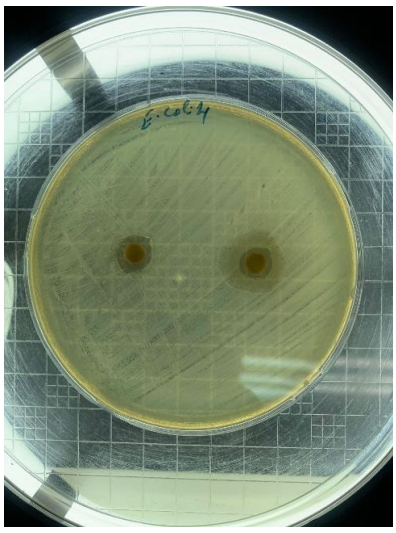
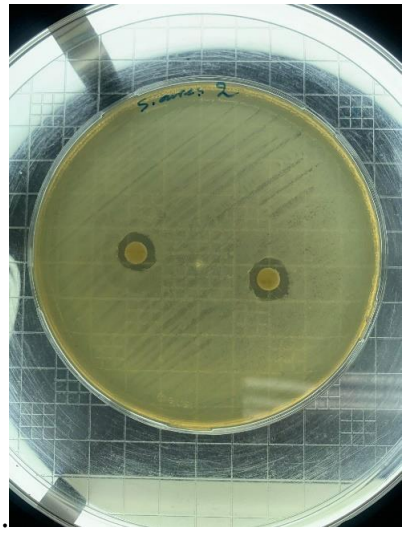
A.  B. 

Fig. 11. Antimicrobial activity against of Dichloromethane extract against *Escherichia coli* ATCC 25922 **(A)**; antimicrobial activity of Butanol extract against *Staphylococcus aureus* ATCC 25923 **(B)**

4. Discussion

The results obtained from the study on the antioxidant activity of *A. horrida* extracts provide valuable insights into the potential health benefits of this plant. The study utilized various antioxidant assays, including DPPH, ABTS, FRAP, and CUPRAC, to evaluate the ability of the extracts to scavenge free radicals and exhibit antioxidant properties. The DPPH test revealed that ethyl acetate (EA) extract demonstrated the highest radical scavenging activity, with an IC_{50} value of 5.44 µg/ml, comparable to the standard antioxidant Torlox. This suggests that AE extract has a strong ability to neutralize free radicals, making it a promising candidate for further research as a natural source of antioxidants. The antioxidant effect observed for AE can be attributed to its high concentration of phenolic compounds, known for their antioxidant properties.

On the other hand, the BU and DC extracts have low phenols content, showed relatively low antioxidant activity, confirming that phenolic compounds are responsible for the observed antioxidant effects. Similarly, in the ABTS assay, the ethyl acetate (AE) extract demonstrated significant antioxidant capacity, with an IC_{50} value of 6.68 µg/ml. This confirms the potential of the AE extract as a potent scavenger of ABTS radicals. The EC, also exhibited a strong antioxidant activity, closely approaching the IC_{50} value of Torlox. SE and ME showed also a significative activity. The variation in antioxidant effectiveness among extracts may arise not only from variations in concentration but also from differences in the composition of phenolic compounds obtained through distinct extraction techniques (Rayan and Elfadil, 2019).

Moving on to the FRAP assay, the EC extract stood out with a notable reducing power, with an $A_{0.5}$ value of 3.28 µg/ml, which was almost equivalent to that of the standard Torlox (3.21 µg/ml). This suggests that the EC extract contains potent electron-donating compounds that contribute to its strong reducing capacity. On the other hand, the BU extract exhibited weaker reducing power, indicating a lower concentration of electron-donating compounds in this extract.

The CUPRAC assay also provided insights into the antioxidant potential of the extracts. The EC extract demonstrated the highest antioxidant activity, closely approaching that of the standard. This reinforces the idea that the EC extract is rich in compounds capable of copper reduction. The other extracts, particularly AE and ES, also showed antioxidant capacity, albeit at slightly lower levels compared to EC. However, all the tested extracts exhibited reducing abilities distinct from that of Torlox, indicating that they might contain a different profile of antioxidant compounds.

Regarding the antidiabetic and anti-inflammatory activities, the results were less promising, as all the extracts showed minimal effects compared to the respective standards (acarbose and diclofenac). This suggests that the tested *A. horrida* extracts might not possess significant antidiabetic or anti-inflammatory properties under the study conditions. Further investigations are warranted to explore the potential of these extracts in other experimental models or in vivo studies.

The antimicrobial activity of the extracts was assessed against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. Among the extracts, DC and BU demonstrated antimicrobial potential, with clear zones of inhibition around the discs. This indicates that these extracts possess compounds with antibacterial properties against the tested strains, making them potential candidates for further exploration as natural antimicrobial agents.

In conclusion, the results of this study highlight the promising antioxidant and antimicrobial activities of *A. horrida* extracts, particularly the ethyl acetate (AE) and ethyl chloride (EC) extracts. These extracts exhibited significant radical-scavenging and reducing abilities, which could be attributed to their high polyphenolic content. However, further investigations are required to fully understand the chemical composition and potential health benefits of these extracts. Additionally, the limited antidiabetic and anti-inflammatory effects suggest that the plant's therapeutic potential in these areas may be limited or influenced by other factors, which necessitates more in-depth research. Overall, this study contributes to the growing body of knowledge on the potential applications of *A. horrida* extracts in the field of natural antioxidants and antimicrobials.

Conclusions

Acacia horrida, a plant belonging to the Fabaceae family of leguminous shrubs found in arid or semi-arid regions of Algeria, has garnered significant research interest.

In this study, six extracts were prepared using different extraction methods and solvent systems to evaluate various biological activities: EC: classical extract, ES: sonication extract, EM: microwave extract, DC: dichloromethane extract, AE: ethyl acetate extract, BU: butanol extract. Quantitative analysis of total polyphenols using the Folin-Ciocalteu method revealed that the AE extract exhibited the highest polyphenol content, followed by ES, EM, EC, and BU extracts. Similarly, the quantification of total flavonoids using the aluminum chloride method demonstrated that the ES extract had the highest flavonoid content, followed by EM, EC, and AE extracts.

The results indicate that the majority of polyphenols and flavonoids in *A. horrida* are moderately polar and soluble in ethyl ether. Among the extraction methods used, ultrasonication proved to be the most efficient compared to maceration and microwave-assisted extraction. Regarding antioxidant activity, the AE extract displayed a robust antioxidant capacity, likely attributed to its rich polyphenol content. Among the tests employed, the DPPH and ABTS radical scavenging assays were the most effective, with the extract values approaching those of the standard. Concerning biological activities, no significant anti-inflammatory or antidiabetic effects were observed in any of the extracts. However, both DC and BU extracts demonstrated antimicrobial (specifically antibacterial) activity against the pathogenic bacteria *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923, respectively. The strength of this antibacterial activity should be further specified in subsequent studies.

Based on these results, the plant extract, could serve as a valuable natural source of antioxidants and antibacterial agents. For future research prospects, the following suggestions are proposed:

- Conduct *in vivo* preclinical studies to validate the in vitro findings.
- Explore the potential use of the studied extracts as antioxidants in agri-food and cosmetic formulations.
- Investigate other parts of the plant, such as flowers and seeds, for potential bioactive compounds.

In conclusion, this study contributes to the understanding of *A. horrida* plant’s medicinal potential and opens avenues for further research on its applications in health and industry.

Acknowledgement

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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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