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Characterization and antibacterial activity of alkaloids and polyphenols extracts from *Haplophyllum tuberculatum* (Forssk.) A. Juss.

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Abstract: *Haplophyllum tuberculatum* is a medicinal plant belonging to the Rutaceae family, is renowned for its various therapeutic properties. This study aims to characterize the alkaloids and polyphenol extracts from this plant and assess their antibacterial activity. Herein, the extraction of polyphenols and alkaloids from this plant was performed by the maceration-method. Folin Ciocalteu's method was used to estimate the total phenolic content, and the qualitative characterization of the two extracts was performed by thin-layer chromatography. Whilst, the antibacterial activity of the two extracts was tested with the disk diffusion method on a solid medium and the minimal inhibitory concentration (MIC) of susceptible bacteria was determined using the agar dilution method. Our results indicate respective yields of 8.39% in polyphenols and 0.37% in alkaloid extracts, while the total phenolic content was estimated to be 74.45 mg GAE/g of dry matter in polyphenolic extract. Thin-layer chromatography analysis allowed choosing the system toluene-acetate-ethanol-concentrated ammonia (40:4:8:3, v/v) to separate *H. tuberculatum* alkaloids, and ethyl acetate-methanol-water (100:13.5:10, v/v) for the separation of its polyphenols. The same analysis detected traces of quercetin, catechin, and rutin in the polyphenolic extract. Our findings demonstrated good antibacterial activity on Gram-positive strains such as *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633, and moderate activity on *Pseudomonas aeruginosa* ATCC 27953, with MICs ranging from 0.625 to 10 mg/mL for alkaloids and from 5 to 20 mg/mL for polyphenols.

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1. INTRODUCTION

The study of plant chemistry continues to be of enduring importance, despite its historical origins. This enduring relevance arises from the fact that the plant kingdom serves as a significant source of a wide array of bioactive molecules that find diverse applications in various industries, such as food production, cosmetics, and pharmaceuticals. Among these compounds, alkaloids and polyphenols play particularly prominent roles (Ferrari, 2002).

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Alkaloids are nitrogenous organic substances, most often of plant origin. They have multiple pharmacological activities and are used as depressants, antispasmodics, vasodilators, and anticancer drugs, but some of them are also used as antimicrobial agents (nicotine, caffeine, morphine, lupine) (Iserin, 2001; Catier & Roux, 2007). However, phenolic compounds constitute a large heterogeneous group of secondary metabolites of plant origin (Sarni-Manchado *et al.*, 2006). The basic structural element is a benzene ring to which one or more hydroxyl groups are linked, free, or committed to another chemical function (ester, heteroside, ether) (Bruneton, 1999). Research on phenolic compounds is very advanced because of their various physiological properties such as anti-allergic, anti-atherogenic, anti-inflammatory, hepatoprotective, antimicrobial, antiviral, anticarcinogenic, antithrombotic, cardioprotective and vasodilatory activities. These actions are attributed to their antioxidant effect (Hoffmann, 2003).

Herbal medicine is increasingly emerging as an alternative for treating various diseases. The situation becomes more concerning after six decades of antibiotic use, as human and animal pathogenic bacteria have reached alarming levels of resistance to several antibiotics (Muylaert & Mainil, 2013). Additionally, it is imperative to employ the appropriate bioassays to assess the biological activities of plants (Tyihák *et al.*, 2008).

Our study focused on *Haplophyllum tuberculatum*, a member of the Rutaceae family. It is found extensively across northern Africa, particularly in the northern Sahara and southern Europe. Its distribution encompasses five distinct regions: Irano-Turanian, Mediterranean, Saharo-Arabian, and Sudano-Zambezian regions (Benchelah *et al.*, 2000). This plant is characterized by large glands containing a potent and unpleasant-smelling essence (Salvo *et al.*, 2011). Traditionally, the plant has been used to treat various conditions such as diabetes, liver diseases, ear infections, and for rheumatic pains (decoction). It is also reputed for alleviating menstrual pain, combating heart diseases, and treating scorpion stings (Hadjadj, 2015; Abdelgaleil *et al.*, 2020).

H. tuberculatum offers versatile usage options. It can be prepared as a boiled mixture or infused in milk as a remedy for stomach aches and bloating. Furthermore, when dried, it is used as a condiment to flavor goat butter and tea. The juice extracted from *H. tuberculatum* leaves is highly sought-after and is particularly used to remove warts and treat skin infections, parasitic diseases, and arthritis (Benchelah *et al.*, 2000; Raissi *et al.*, 2016). Also, the leaves of this plant infused in vinegar exhibited activity on the central nervous system. The mixture can be administered to children for the treatment of convulsions and other nervous disorders (Al-Said *et al.*, 1990).

H. tuberculatum has proven its effectiveness in traditional medicinal practices, which makes the study of its various biological activities particularly interesting and likely to require extensive research. Among these studies, Abou-Zeid *et al.* (2014) and Al-Saeghi *et al.* (2022) have demonstrated a remarkable antibacterial effect of different extracts from the aerial parts of the plant against a wide range of Gram-negative and Gram-positive bacteria, with inhibition zones of 6-12 mm and 6-20 mm, respectively. Moreover, the study carried out by Hamdi *et al.* (2021) revealed the cytotoxicity and antiviral activities of *H. tuberculatum* essential oils against Cocksackie viruses B3 and B4.

Our research is, therefore, part of this perspective and aims to extract alkaloids and polyphenols from the aerial part of *H. tuberculatum* using the maceration method, characterize these compounds by thin layer chromatography (TLC), and evaluate their antibacterial activity.

2. MATERIAL and METHODS

2.1. Plant Material

The plant material used is the aerial part of *H. tuberculatum* plant collected from Algerian fluent, in the region of Tsabit (Adrar, Algeria), during the flowering period (February-May). The plant was dried for two weeks in the open air at room temperature and protected from light.

2.2. Microbial Strains

The microbial support used is represented by Gram-positive and Gram-negative bacteria: *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27953, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, *Klebsiella pneumoniae* ATCC 700603 (laboratory strains supplied by SAIDAL-antibiotic and the Pasteur Institute of Algeria), *Klebsiella* sp. and *Proteus* sp. (strains obtained from urine cultures of hospitalized patients, provided by Bacteriology Laboratory, Hospital Krim Belkacem, Tizi Ouzou, Algeria).

2.3. Preparation of *H. tuberculatum* Extracts

The preparation of the extracts was carried out by maceration of the aerial parts of the plant. The alkaloid extract was prepared according to the method of Bruneton (1999), the powder of the plant was dissolved in a solution of hydrochloric acid (HCl 1N) and left to stir for two hours. After filtration and degreasing, the solution was placed in a separatory funnel then basified with ammoniac and extracted with chloroform. Finally, it was washed and dried over anhydrous sodium sulfate before drying in a rotary evaporator under a vacuum at 40 °C.

The extraction of total polyphenols was performed as described by Owen *et al.* (1999) through maceration of the powder in methanol for 3 days under magnetic stirring, followed by filtration. The obtained filtrate was evaporated at 40 °C using a rotary evaporator. The extract was weighed and stored at 4 °C for future uses.

2.4. Yields of Extraction and Total Polyphenols Assay

The quantitative estimation of the alkaloids and polyphenols extracts obtained from the aerial part of *H. tuberculatum* was carried out by yield determination, which is based on the ratio between the mass of the crude extract in the dry state and that of the plant material used.

Moreover, the total phenolic content of the extract was estimated using Folin Ciocalteu's method, as described by Wong *et al.* (2006), with Gallic acid as the standard. A volume of 1 mL of total polyphenolic extract of *H. tuberculatum* and different concentrations of Gallic acid was added to 5 mL of distilled water and 0.5 mL of Folin-Ciocalteu reagent (10 times diluted in water). After 3 min, 2 mL of sodium carbonate Na₂CO₃ at 20% (w/v) was added. The solutions were then incubated in the dark for 30 min at room temperature. The absorbance reading was taken at 760 nm against a blank containing ethanol instead of Gallic acid. The result was expressed as milligrams equivalent of Gallic acid per gram of dry matter (mg GAE/g DM).

2.5. Detection of *H. tuberculatum* Compounds

The qualitative analysis of extracts was carried out by thin layer chromatography (TLC), where 10 µL of each extract was deposited at 1.5 cm from the lower edge of the plate. After drying, the plate emerged into the glass tank previously saturated with the appropriate eluent. To select the best solvent system that allowed a good separation of compounds for the two extracts studied, several mini-TLCs were carried out using aluminum plates covered with Silicagel 60 F254 (Merck) and different solvent systems (Table 1).

After development and drying, the plates were observed under UV at 365 nm, then revealed by the appropriate reagents allowing the detection of the separated compounds. Dragendorff's reagent was used to reveal alkaloids which appear in the form of orange spots, while polyphenols were revealed by ammonia vapors which give brown stains.

Table 1. Composition of the solvent systems tested (v/v) for the separation of polyphenols and alkaloids of *H. tuberculatum* by TLC.

Alkaloids	Polyphenols
S1: Chloroform-Methanol (9:1)	S1: Ethyl acetate-Formic acid-Acetic acid glacial-Distilled water (100:11:11:27)
S2: Chloroform-Acetone (9:1)	S2: Ethyl acetate-Methanol-Distilled water (100:13.5:10)
S3: Hexane-Ethyl acetate (8:2)	S3: Butanol-Acetic acid-Distilled water (4:1:5)
S4: Chloroform-Ethyl acetate-Acetone (5:4:1)	S4: Chloroform-Methanol-Distilled water (1:1:0.5)
S5: Ethyl acetate-Methanol-Distilled water (77:13:10)	
S6: Chloroform-Methanol-Concentrated ammonia (90:9:1)	
S7: Diethylether-Methanol-Concentrated ammonia (44:5:1)	
S8: Toluene-Acetone-Ethanol-Concentrated ammonia (40:4:8:3)	

2.6. Evaluation of The Antibacterial Activity

The disk diffusion method on solid medium as described by Balouiri *et al.* (2016) was used. Discs of filter paper (N^o4) of 9 mm diameter impregnated with 60 μ L of ethanol solution containing each extract corresponding to 3 mg per disc, also a disc containing the same volume of ethanol 95° (as control negative), were dried and then placed on Mueller Hinton agar previously seeded with bacterial suspension.

The Petri dishes were kept at 4 °C for three hours to promote the diffusion of extracts (Bansemir *et al.*, 2006). Afterward, they were incubated at 37 °C for 24 h. Reading was performed by measuring the inhibition diameter. The results were expressed in mm, and bacteria that showed a clear zone of inhibition were considered sensitive.

An extract is considered active when it produces an inhibition zone around the disk, and bacterial sensitivity to different compounds is classified based on the diameter of the inhibition zones as follows: $\emptyset \leq 8$ mm: non-sensitive strain; $9 \leq \emptyset \leq 14$ mm: sensitive strain; $15 \leq \emptyset \leq 19$ mm: highly sensitive strain; $\emptyset \geq 20$ mm: extremely sensitive strain (Ponce *et al.*, 2003).

The experiments were made in duplicate and repeated three times. The effect of each extract was compared to Amikacin (30 μ g), Kanamycin (30 UI), Oxacillin (5 μ g), amoxicillin (25 μ g), Penicillin G (6 μ g), Amoxicillin + Clavulanic acid (25 μ g), Chloramphenicol (30 μ g), Colistin (50 μ g), Vancomycin (30 μ g), and Doxycyclin (30 UI).

Statistical analysis was performed using XLSTAT 7.5.2. (2007). Data were analyzed statistically using one-way analysis of variance (ANOVA) (for $p < 0.05$; differences were considered to be statistically significant).

2.7. Determination of The Minimum Inhibitory Concentration (MIC)

The MIC was estimated by the dilution method on a solid medium, as described by Amhis *et al.* (2001), with few modifications. The technique consists of adding, in sterile test tubes, 9 mL of Mueller Hinton agar in superfusion, with different quantities of polyphenolic and alkaloid extract of *H. tuberculatum*. The final concentrations of each extract in the culture medium were: 0.625, 1.25, 2.5, 5, 10, and 20 mg/mL. Once mixed, the content of each tube was poured into a Petri dish. After solidification, each dish was inoculated simultaneously with several bacterial strains in the form of parallel strips. A control dish containing 1 mL of ethanol 95° and 9 mL of Mueller Hinton agar was inoculated in the same way. The MIC is given by the first concentration, which removes any apparent culture on the agar.

3. RESULTS

3.1. Yield of Extracts and Total Polyphenols Content

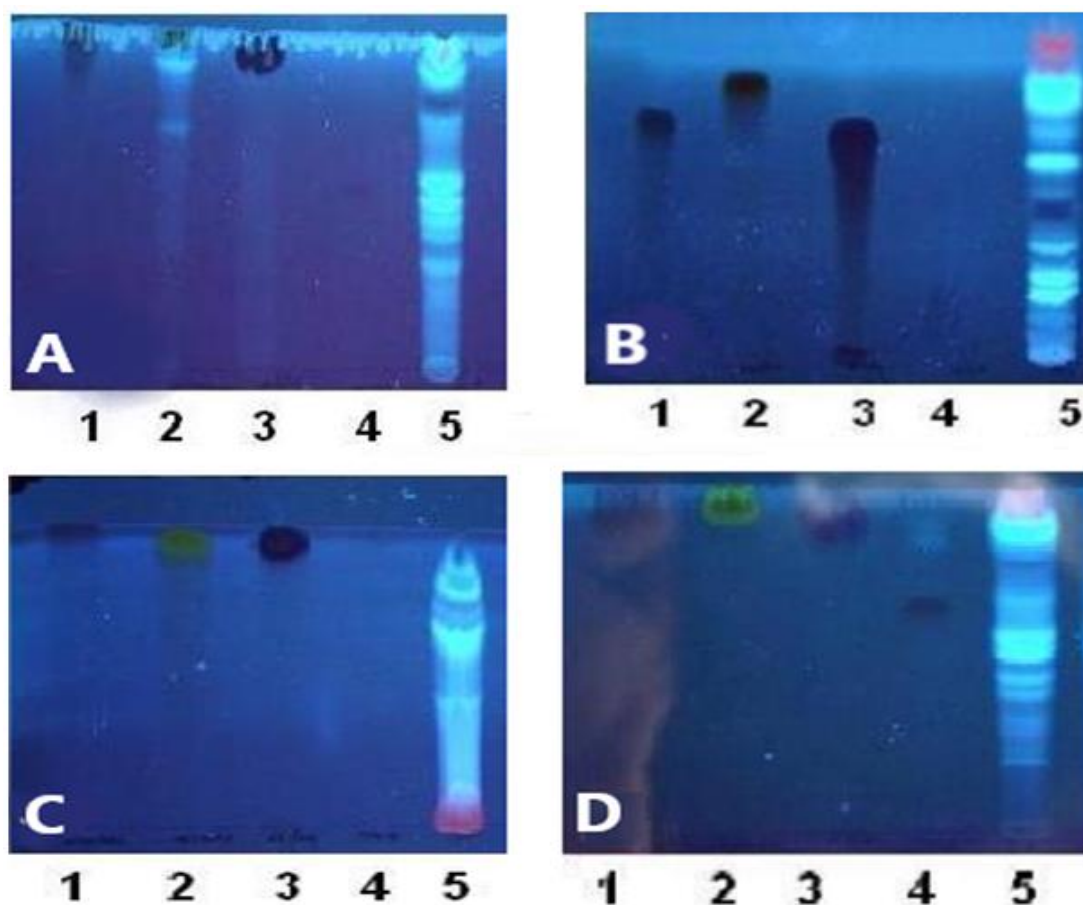
The extraction of polyphenols and alkaloids of *H. tuberculatum* by maceration method showed that the plant is rich in phenolic and alkaloid compounds. The yield was $8.39 \pm 0.04\%$ for total polyphenols against only $0.37 \pm 0.01\%$ for alkaloids. Using the Folin Ciocalteu's reagent, the content of polyphenols was estimated at 74.45 ± 1.43 mg GAE/g DM.

3.2. Qualitative Analysis of *H. tuberculatum* Extracts

3.2.1. Characterization of the total polyphenolic extract

Figure 1 shows the chromatograms obtained with the various solvent systems as the mobile phase under a UV lamp at 365 nm. The results reveal that the solvent system S3 (n-butanol, acetic acid, distilled water (4:1:5)) provided poor separation for the compounds of *H. tuberculatum* extract, as well as for the standard molecules (Figure 1, C). The solvent system S1 (ethyl acetate, formic acid, glacial acetic acid, distilled water (100:11:11:27)) enabled a good separation of the total polyphenolic extract and well-defined spots for the standard molecules (Figure 1, A). In contrast, solvent system S2 (ethyl acetate, methanol, distilled water (100:13.5:10)) yielded a satisfactory separation of the crude polyphenolic extract, showing distinct spots and efficient migration of standard molecules (Figure 1, B). Similar observations were made with solvent system S4 (chloroform, methanol, distilled water (1:1:0.5)), but with a lower number of spots in the crude polyphenolic extract (Figure 1, D).

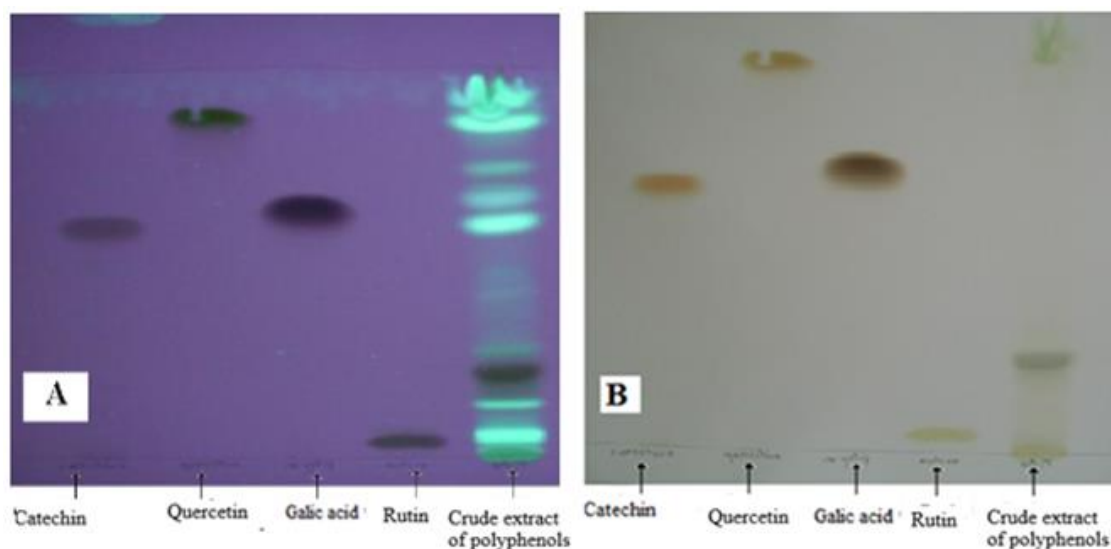
Figure 1. Revelation under UV at 365 nm of the chromatograms of polyphenolic extract from *H. tuberculatum* and standard molecules obtained with the four solvent systems tested. **1:** Catechin, **2:** Quercitin, **3:** Gallic Acid, **4:** Rutin, **5:** Total polyphenols extract. **A, B, C** and **D:** Chromatograms developed with the solvent system S1, S2, S3 and S4, respectively.



Based on these findings, we selected the combination of ethyl acetate, methanol, and distilled water (100:13.5:10) as it provided the best separation of the total polyphenolic extract of *H. tuberculatum* and the standard molecules used.

Subsequently, a new separation was performed on an entire 20 cm × 20 cm plate to ensure a good identification of the different spots and their colors (Figure 2). The results revealed that the total polyphenols extracted by maceration in methanol exhibited blue or brown fluorescence bands. Exposure of the chromatogram to ammonia vapors unveiled the presence of darker brown-colored spots. According to the obtained observations and with comparison to standard molecules, traces of quercetin, catechin, and rutin were found in our plant's polyphenolic extract.

Figure 2. Chromatograms of the polyphenolic extract of *H. tuberculatum* and the standard molecules separated with the solvent system S2: ethyl acetate-methanol-distilled water (100:13.5:10). **A:** Observation under UV at 365 nm. **B:** Revelation with ammonia vapors.



3.2.2. Characterization of the alkaloid extract

The results of observation under UV at 365 nm (Figure 3) of *H. tuberculatum* alkaloids carried out with the eight solvent systems revealed several fluorescent spots, the number of which varied according to the composition of the mobile phase. Solvent systems S1, S2, S4, S6, and S8 (composition: see Table 1) were able to cause the alkaloid extract of *H. tuberculatum* with varying degrees. The best separation was observed with the S8 system, which gave the greatest number of fluorescent spots. However, the chromatograms of the solvent systems S3, S5, and S7 (composition: see Table 1) showed streaks.

After revealing the chromatograms with Dragendorff's reagent, the results showed characteristic orange spots of alkaloids in all chromatograms (Figure 4). Nevertheless, it was the S8 system (toluene-acetate-ethanol-concentrated ammonia 40:4:8:3) that gave the maximum number of these spots (about ten spots), thus suggesting that *H. tuberculatum* is quite rich in alkaloid compounds of different natures.

Figure 3. Chromatograms of the alkaloid extract of *H. tuberculatum* observed under UV at 365 nm and obtained with the different solvent systems.

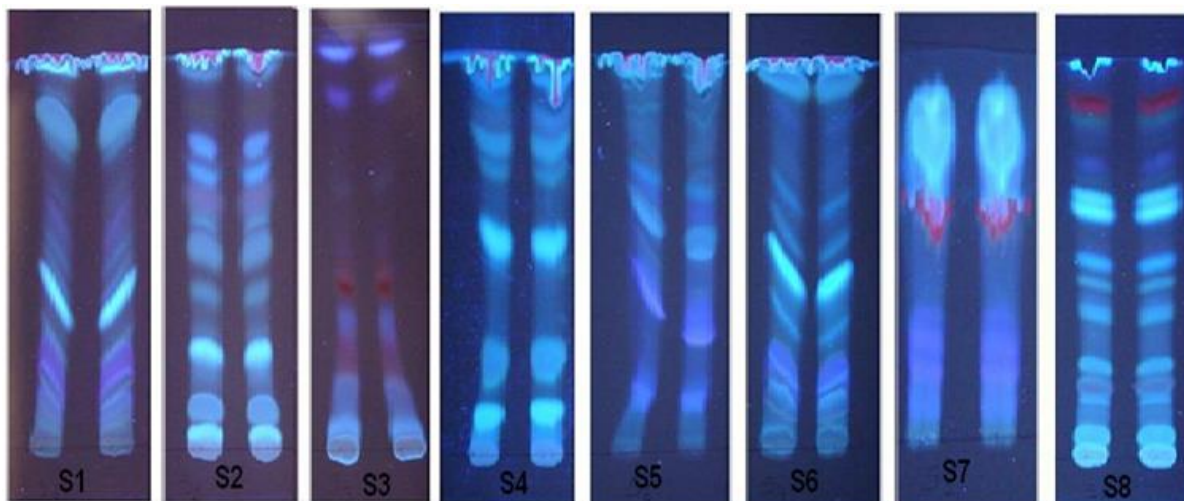
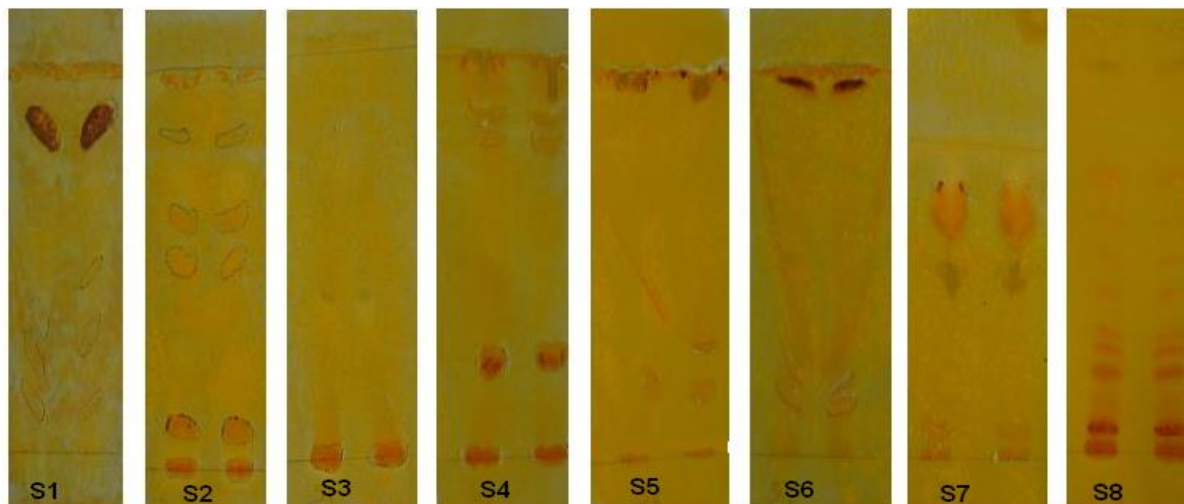


Figure 4. Chromatograms of the alkaloid extract of *H. tuberculatum* revealed by Dragendorff 's reagent and obtained with the different solvent systems.



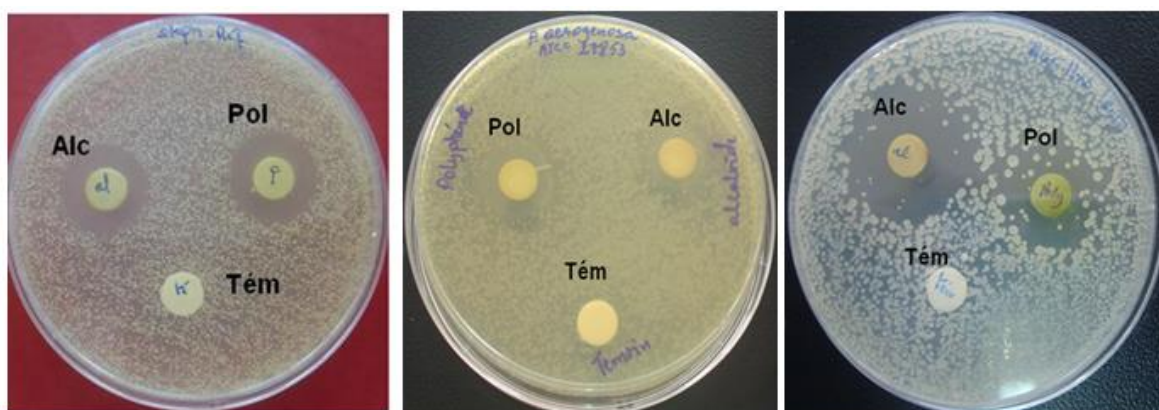
3.3. Antibacterial Activity of *H. tuberculatum* Extracts

The results (Table 2 and Figure 5) show that some bacterial strains are sensitive to both extracts, the Gram-positive strains represented by *S. aureus* ATCC 25923 and *B. subtilis* ATCC 6633 with halos of inhibition around 19 ± 1.00 mm and 25.66 ± 4.04 mm, respectively, for alkaloids, and 20.66 ± 1.52 mm and 22 ± 2.00 mm for polyphenols. The sensitive Gram-negative strains were represented by *P. aeruginosa* ATCC 27953 with zones of inhibition of 14 ± 1.00 mm for alkaloids and 17 ± 2.00 mm for polyphenols. Whereas, the other Gram-negative strains tested: *E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603, *Proteus* sp. and *Klebseilla* sp. were all resistant.

Table 2. Diameter of inhibition zones induced by *H. tuberculatum* extracts (3 mg of extract/disc) and Amikacin (30µg) as positive control using disk diffusion method.

Strain	Diameter of inhibition zones (mm)		
	Polyphenols	Alkaloids	Amikacin (30µg)
<i>S. aureus</i> ATCC 25923	20.66 ± 1.52	19.00 ± 1.00	24.33 ± 2.08
<i>P. aeruginosa</i> ATCC 27953	17.00 ± 2.00	14.00 ± 1.00	22.16 ± 1.25
<i>B. subtilis</i> ATCC 6633	22.00 ± 2.00	25.66 ± 4.04	34.00 ± 2.00
<i>E. coli</i> ATCC 25922	/	/	NT
<i>K. pneumoniae</i> ATCC 700603	/	/	NT
<i>Proteus</i> sp.	/	/	NT
<i>Klebsiella</i> sp.	/	/	NT

/: Absence of inhibition, ND: not tested.

Figure 5. The appearance of cultures and inhibition zones in the presence of polyphenolic and alkaloid extracts from *H. tuberculatum*, obtained by the disk diffusion method on Mueller Hinton agar (**Tém**: negative control, **Pol**: total polyphenols, **Alc**: alkaloids).*S. aureus* ATCC25923*P. aeruginosa* ATCC27953*B. subtilis* ATCC6633

In addition, several antibiotic tests were carried out to evaluate the sensitivity of the three strains. The results of the antibiogram demonstrated that Amikacin (30 µg) exhibited the highest effect against all sensitive strains to alkaloid and polyphenol extracts from the plant *H. tuberculatum*. Therefore, it was chosen as the positive control, where its antibiotic effect was proved to be superior to that of the tested plant extracts. The inhibition zones of Amikacin are mentioned in Table 2. Analysis of variance with a confidence level of 95% ($p < 0.05$) revealed that the difference between the effect of polyphenolic and alkaloid extracts of *H. tuberculatum* and the control (Amikacin) is significant for the three sensitive strains.

3.4. The Minimum Inhibitory Concentration of Alkaloid and Polyphenolic Extracts

The results of the MIC (Table 3) show that the alkaloids of *H. tuberculatum* were more active than its polyphenols, with MICs between 0.625 to 10 mg/mL for alkaloids (Figure 6) and from 5 to 20 mg/mL for polyphenols (Figure 7). Moreover, *B. subtilis* ATCC 6633 was the most sensitive strain to both extracts, followed by *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27953.

Table 3. MICs of polyphenols and alkaloids extracts from *H. tuberculatum* against some bacterial strains.

Bacterial strains	MIC (mg/mL)	
	Polyphenols	Alkaloids
<i>S. aureus</i> ATCC 25923	10.0	0.625
<i>P. aeruginosa</i> ATCC 27953	20.0	10.00
<i>B. subtilis</i> ATTC 6633	5.00	0.625

Figure 6. The appearance of bacterial cultures on Mueller Hinton agar in the presence of increasing doses of *H. tuberculatum* alkaloids. (**B**: *B. subtilis* ATCC 6633, **St**: *S. aureus* ATCC 25923, **Ps**: *P. aeruginosa* ATCC 27953). **1**: MH agar + alkaloids 0 mg/mL, **2**: MH agar + alkaloids to 0.625 mg/mL, **3**: MH agar + alkaloids to 1.25 mg/mL, **4**: MH agar + alkaloids to 2.5 mg/mL, **5**: MH agar + alkaloids to 5 mg/mL, **6**: MH agar + alkaloids to 10 mg/mL.

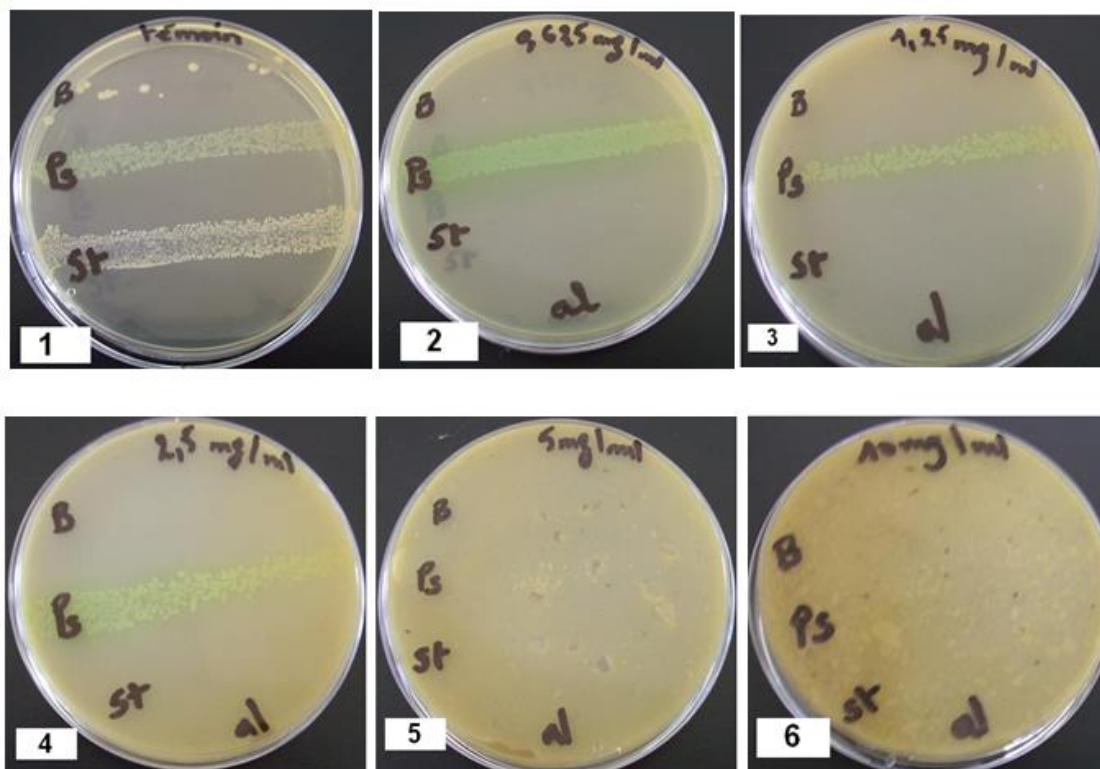
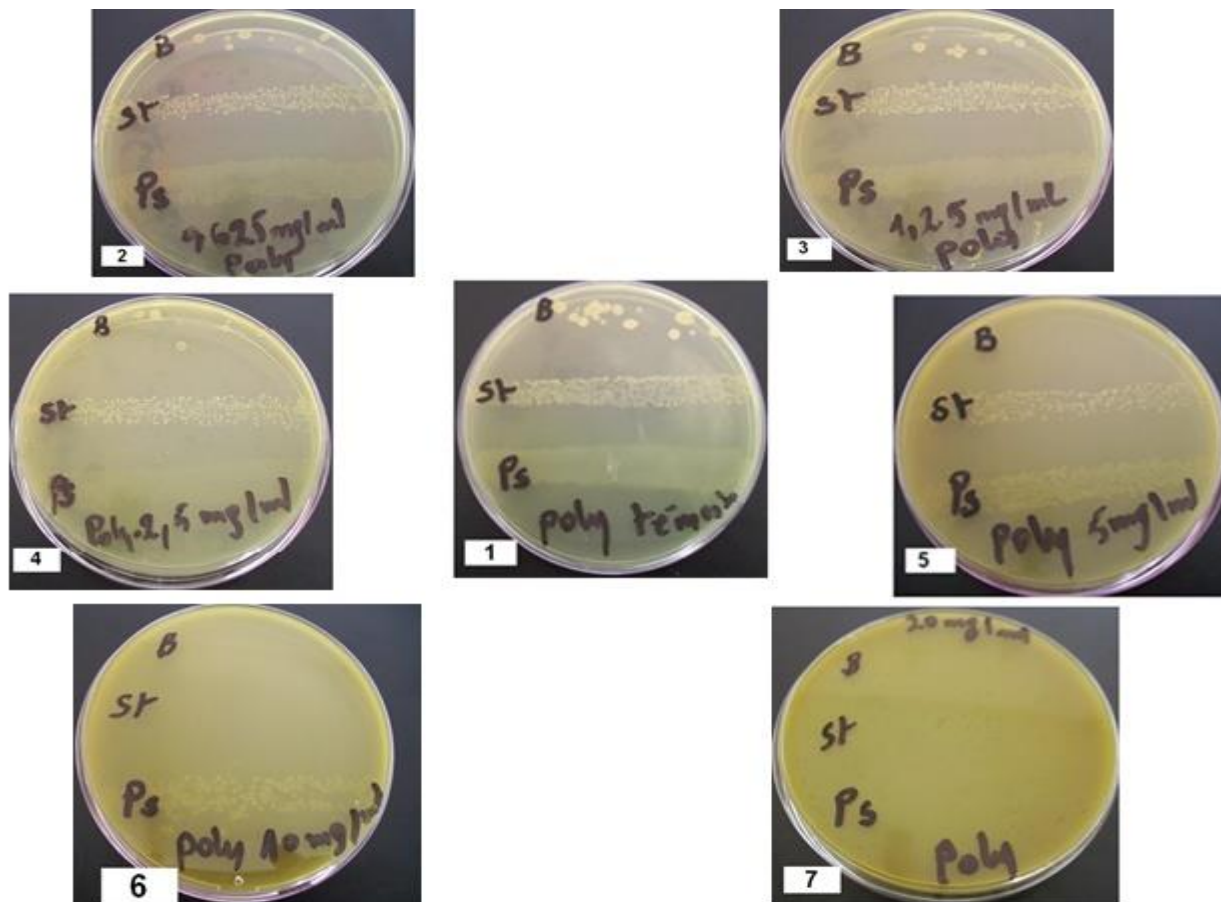


Figure 7. The appearance of bacterial cultures on agar Muller Hinton (MH) in the presence of increasing doses of *H. tuberculatum* polyphenols. (B: *B. subtilis* ATCC 6633, St: *S. aureus* ATCC 25923, Ps: *P. aeruginosa* ATCC 27953). 1: MH agar + polyphenols at 0 mg/mL, 2: MH agar + polyphenols to 0.625 mg/mL, 3: MH agar + polyphenols to 1.25 mg/mL, 4: MH agar + polyphenols to 2.5 mg/mL, 5: MH agar + polyphenols to 5 mg/mL, 6: MH agar + polyphenols to 10 mg/mL, 7: MH agar + polyphenols to 20 mg/mL.



4. DISCUSSION and CONCLUSION

According to Al-Brashdi *et al.* (2016), the aerial part of *H. tuberculatum* is rich in phenolic compounds that could play a vital role in discovering natural antioxidants. The same authors have observed that the most polar fractions (methanol) have the highest content of phenols (561.22 mg GAE/g), which is higher than the one found during our work (74.45 ± 1.43 mg GAE/g DM). These variations in results were related to several factors: climatic and environmental factors (region, soil, salinity, etc.), genetic factors, storage duration, period and stage of the plant development (Miliauskas *et al.*, 2004; Bouzid *et al.*, 2011; Touati *et al.*, 2018). In addition, Lee *et al.* (2003) indicated that the extraction and quantification methods can influence the estimation of the total phenols content.

The characterization of polyphenolics and alkaloids of *H. tuberculatum* by thin-layer chromatography and their observation under UV at 365 nm revealed blue and brown fluorescence bands. According to Dohou *et al.* (2003) and Diallo (2005), the bands of blue fluorescence would correspond to phenol acids or the presence of coumarins, while those colored brown would correspond to flavonols and flavones.

The work of Abdelkhalek *et al.* (2020) proved the presence of gallic acid, catechin, quercetin and rutin in the crude ethanolic extract of *H. tuberculatum* following High Performance Liquid Chromatography (HPLC) analysis, which confirms our results.

The revelation of chromatograms using ammonia vapors for polyphenols and Dragendorff's reagent for alkaloids showed several characteristic spots of the two secondary metabolites. Indeed, the work of Hamdi *et al.* (2018) showed the presence of phenols, tannins, alkaloids, flavonoids, terpenoids, sterols, cardiac-glycosides, saponosides, lipids and fixed oils in the crude methanolic extract.

Furthermore, the work performed by Al-Shamma (1979), Sheriha (1984) and Al-Yahya *et al.* (1992) on the same plant showed that the alkaloids of this plant are very variable; and represented by haplotubinone, haplotubin, tuberine, tubacetin, tubasenicin, quinolines, flindersin, skimianine and evoxin.

Concerning the antibacterial activity of the two extracts of the plant, it was found that all the strains sensitive to the alkaloid extract are simultaneously sensitive to the polyphenolic extract. However, the degree of sensitivity is variable, where the alkaloids are more effective. This can be explained by the presence of common substances between the two extracts. Moreover, the presence of phenolic acids was confirmed in the alkaloid extract by the appearance of blue bands after its observation under UV. Likewise, the polyphenolic extract may contain alkaloids in the form of soluble salts in methanol.

Regarding the sensitivity of Gram-positive bacteria compared to Gram-negative towards plant extracts, several studies have demonstrated this effect (Hayouni *et al.*, 2007; Shan *et al.*, 2007; Falleh *et al.*, 2008; Çolak *et al.*, 2009). This and according to the same authors, is due to differences in the outer layer composition of bacteria. The Gram-negative strains have more, outer membrane, which is composed of phospholipids, proteins and lipo-polysaccharides, impermeable to most molecules.

The comparison of our results with those of previous studies showed that the antibacterial effect of polyphenolic and alkaloid extracts of *H. tuberculatum* is partially consistent with the work of Sheriha (1984) and Al-Rehaily (2001) performed on the same plant. These authors reported that Tuberin isolated from this plant collected in Iraq exerts an antibacterial effect against *S. aureus* and *E. coli* with doses of 0.10 to 1.0 mg/mL. We also noted that the phenolic and alkaloid substances of this plant have less effect on Gram-negative bacteria than its essential oils, where the work carried out by Al-Burtamani *et al.* (2005), showed that the essential oils of *H. tuberculatum* inhibit the growth of *E. coli* and *Salmonella choleraesuis*.

The phytochemical study established by Abdelgaleil *et al.* (2020) led to the isolation of three alkaloids (skimmianine, vulcanine, and evoxine) from the aerial parts of *H. tuberculatum*. Skimmianine was among the most potent inhibitors against the phytopathogenic bacteria: *Rhizobium radiobacter*, *Ralstonia solanaceum* and *Pectobacterium carotovorum*, with MIC value of 62.5 µg/mL.

Compared to other species of the *Rutaceae* family, the work of Singh *et al.* (2020) shows the antibacterial efficacy of methanolic extract from *Zanthoxylum armatum* leaves against Gram-positive and Gram-negative bacterial strains with an inhibition zone of 17.67 mm for *S. aureus* and 12.33 mm for *E. coli*. According to work published by Pavić *et al.* (2019), the best antibacterial activity of *Ruta graveolens* extracts was observed against *P. aeruginosa* with the lowest MIC (62.5 µg/mL). While the highest MIC (125 µg/mL) was observed against *E. coli*, *B. subtilis* and *S. aureus*.

Indeed, alkaloids are known for their high antibacterial effect. The mechanism action of antibacterial alkaloids intervenes through blocking cell division, by preventing the synthesis of microtubules and mitotic spindle formation, which is essential for cell division (Karou, 2005).

The antimicrobial activity of polyphenols may involve complex mechanisms such as inhibition of cell walls, cell membranes, protein synthesis and nucleic acid metabolism (Aboshora *et al.*, 2014). According to Daglia (2012), flavonols have antibacterial activity

against several Gram-positive bacteria. At equivalent concentrations, some flavonoids have greater antibacterial activity than tetracycline or vancomycin. Similarly, the mechanism of polyphenol toxicity against microorganisms may be related to inhibition of hydrolytic enzymes (proteases and carbohydrases) or other interactions to inactivate adhesion proteins, and cell envelope transport proteins, no specific interactions with carbohydrates, among others (Alvarez et al., 2010).

In conclusion, the present research confirms that *H. tuberculatum* is more enriched in polyphenols than in alkaloids. Qualitatively, thin-layer chromatography showed that this plant contains a wide range of alkaloids and polyphenols, revealed by several spots on the chromatograms. The antibacterial activity was interesting and effective with both extracts, especially on Gram-positive strains. Nevertheless, on Gram-negative strains, the effect was observed only with *P. aeruginosa* ATCC 27953. However, the other Gram-negative strains such as *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Proteus* sp., and *Klebsiella* sp., were all resistant to both extracts. Our study showed that *H. tuberculatum* extracts can be used as promising new sources of antibacterial molecules in the treatment of bacterial infections. Therefore, purification and identification of both extracts by instrumental techniques would be necessary.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship contribution statement

All authors contributed to the study conception and design. The plant was provided by **Fatma Acheuk**. Material preparation, experiments and data analysis were performed by **Djamila Djouahra-Fahem**. The first draft of the manuscript was written by **Djamila Djouahra-Fahem**, **Souhila Bensmail** and **Razika Bouteldja**. **Fethia Fazouane** and **Fatma Acheuk** supervised the work. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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