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Ethnobotanic study, phytochemical screening, antioxidant and antibacterial activities of *Tapinanthus pentagonia*

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ABSTRACT

This study shows that Tapinanthus pentagonia, phanerogam parasite of the family Loranthaceae is widespread in Mauritania and is also used by the Mauritanians to relieve conditions such as infertility, jaundice and kidney stones which attests of its medicinal properties. This phanerogam is also much sought after by livestock. In addition, our phytochemical study on the leaves of T. pentagonia parasite of Acacia tortilis shows the presence of flavonoids, tannins, ellagic acid, proanthocyanidols of saponins, sterols, polyphenols, reducing sugars and terpenes. We have also shown that the presence and distribution of these metabolites known to be responsible for the antioxidant activity of plants, in T. pentagonia are closely related to the nature of the host. The antibacterial activities of the aqueous extracts revealed activity against S auere at different degrees.

Keywords: Ethnobotany, *Tapinanthus pentagonia*, secondary metabolites, Antibacterial activity, antioxidant activity.

INTRODUCTION

The family *Loranthaceae* belongs to the class *Dicotyledonous.* In Africa, this family includes several types, the most common is *Tapinanthus Blume* (also called African mistletoe, as compared to European mistletoe, Viscum gender) [1]. In Mauritania, It was shown that gender *Tapinanthus* is represented by two species: (. A. Rich) Tapinanthus globiferus Tiegh V. and T. pentagonia (DC.) V. Tiegh. Of these taxa, T. pentagonia (DC.) V. Tiegh is the epiphytoide parasite of Acacia which is the most widespread in Mauritania and causes varying damage to its guests [2].

The most attacked hosts by T. pentagonia (DC.) V. Tiegh in Mauritania are especially Acacia senegal (L.) Willd. and A. tortilis (Forsk.) Hayne subsp. raddiana (Savi) Brenan var. raddiana (Mimosaceae), and to a lesser extent, Combretum glutinosum Perr. (Combretaceae) Capparis decidua (Forsk.) Edgew. (Capparaceae), Calotropis procera Ait. and Leptadenia pyrotechnica (Forsk.) Decne (Asclepiadaceae) [3].

In ethnobotany and despite the damage caused to their hosts, *Tapinanthus* are eaten by camels [4] as well as by sheep and goats and farmers cut the tufts usually located high at the crown of the host [3]. Similarly, the leaves of

these flowering plants are used to make bottles for keeping fresh water in desert waterproof. Furthermore, the medicinal properties of these plants, considered medical magic, are very numerous in Africa: for example *Tapinanthus sp*, are used by the Hausa Fulani tribes (Nigeria) to treat human and animal diseases such as cancers, infections due to injuries and gastrointestinal diseases [5-7]. Other African populations use it against infertility, impotence [8], amenorrhea [9] , respiratory diseases, [8-10], mental disorders [8], spells, body aches, fatigue, phlebitis, rickets, rheumatism fever and jaundice [9].

On the other hand, the lectin and mitogenic properties of the family *Loranthaceae* and the ability of a large number of plants to produce mitogenic substances together with their antibiotic properties [11] would inspire further studies on these phanerogams, especially *Tapinanthus pentagonia* in order to identify phytochemicals that have a large economic and medicinal interest.

In the present work, we report on ethnobotanical surveys on *Tapinanthus sp*. among traditional herbalists in Mauritania and show that they are used among others against jaundice, kidney stones and are considered stomachic, diuretic and liver tonics. The presence of secondary metabolites, investigated through a photochemical study of this plant, confirms its antioxidant activity. Our results suggest also that the aqueous extracts of *T. Pentagonia* have antibacterial activity against two bacterial strains.

EXPERIMENTAL SECTION

2.1. Ethnobotanical study

The survey was conducted using forms distributed to various interviewees: traditional healers, shopkeepers, maids and farmers. The record collections were performed directly at the time of the visit by the researcher or by examination.

2.2. Plant material

Leaves *Tapinanthus pentagonia* were harvested in December 2013 in the province of Tagant east of Mauritania (Gabbou zone, extending the Tamourt In Naaj).

The leaves were dried in the open air in the shade, and then finely ground. The powder of the leaves of *T. pentagonia* obtained (200 g) are used for different tests and for the phytochemical antioxidant and antibacterial tests. The plant was identified by the botanist Pof BOUMEDIANA at Ecole Normale Superieure (ENS), Nouakchott, Mauritania.

2.3. Equipment

The weighing is carried out using a scale Denver TL-series, the solvents were evaporated by a rotary evaporator Type Lab-X Rota S-300 equipped with a vacuum pump and a UV-vis spectrophotometer was used to measure the optical density.

2.4. Qualitative phytochemical screening

The extraction methods are simple: maceration and infusion at room temperature. Phytochemical tests were performed to detect secondary metabolites according to standard protocol characterization [12-14], Alkaloids, saponins, tannin, ellagic acid, flavonoids, proanthocyanidins and quinones were characterized according to the experimental procedures described by Trease-Evans (1987) [12,], Sofowora (1982) [13] and Harborne (1973); [14] sterols and terpenes were highlighted by the reactions of Lieberman-Burchard and Salkowski. [13].

The result of each test is qualitatively and phytochemically expressed by the sign (+) positive and (-) negative.

2.4.1. The Tannins

- Tannins were identified by gelatine 5 mL of aqueous extract + 2 mL gelatin were mixed in a test tube, observation of a white precipitate characterizes tannins.
- Gallic tannins were identified by adding FeCl3; the addition of 5 drops of 2% FeCl3 on 5 mL of the aqueous extract showed the appearance of a deep blue-black color indicating the presence of gallic tannins. An alcoholic solution of gallic acid is used as a standard

2.4.2. The saponins

Saponins were highlighted by stirring 10 ml of the aqueous extract in a test tube. The tube was stirred for 15seconds and then left to stand for 15 min. A height of persistent foam, 1 cm higher indicates the presence of saponins.

2.4.3. Proanthocyanidols
Proanthocyanidols were identified by heating to boiling a mixture of 2 mL of the aqueous extract and 2 mL of reagent (concentrated HCl 20 mL + 20 mL butanol), the appearance of a red color indicates the presence of proanthocyanidins.

2.4.4. Ellagic acid
Ellagic acid was identified by mixing a 2 mL test tube of the alcoholic extract, , in a test tube with 5 5% acetic acid and 5 drops of sodium nitrite ($NaNO_2$) to 5%. The mixture is deposited during 40 min, the appearance of a dark brown color reveals the presence of ellagic acid.

2.4.5. Flavonoids
To confirm the presence of the flavonoids, the 'cyanidin' reaction 'was used. Two (2) mL of each extract was evaporated and the residue was taken up in 5 mL of hydrochloric alcohol diluted 2 times. Adding 2-3 magnesium chips leads to an exothermic reaction and then a pink-orange or violet color (purple) was observed. The addition of 3 drops of isoamyl alcohol intensified this coloration which confirmed the presence of flavonoids. An alcoholic solution of quercetin was used as a standard.

2.4.6. Sterols and terpenes
To identify sterols and terpenes, we used the reagent LIEBERMANN. Indeed, 5 mL of plant extract was evaporated to dryness in a water bath. The residue was dissolved in 1 mL of hot acetic anhydride. We added 0.5 mL of concentrated sulfuric acid to triturate. The appearance at the interphase, a purple and purple ring, turning blue then green, indicates a positive reaction. This test was performed with a chloroform solution of cholesterol witness

2.4.7. Alkaloids
To highlight alkaloids, Dragendorff reagent (reagent for iodobismuthate) and Bouchardat (iodoioduré reagent) were used. Indeed, 6 mL of the solution was evaporated to dryness. The residue is taken up in 6 mL of alcohol at 60 ° C. The addition of 2 drops of Dragendorff reagent to the alcoholic solution causes a precipitate or an orange color. The addition of 2 drops of reagent Bouchardat to the alcoholic solution causes a precipitate of reddish-brown color and showed the presence of alkaloids.

2.4.8. Quinones
To highlight quinones, we used the reagent BORNTRAEGEN. 2 mL of the extract was evaporated to dryness. The residue was triturated in 5 ml of hydrochloric acid 1/5. The triturate was then heated in a water bath for 30 min. After cooling, it was extracted with 20 mL of chloroform. Ammonia diluted 2-fold (0.5 mL) was added to the chloroform solution. A red or purple color indicated the presence of quinones.

2.4.9. Polyphenols
To highlight the polyphenols, the reaction with ferric chloride ($FeCl_3$) was used. Thus, 2 ml of each solution was added a drop of alcohol solution of 2% ferric chloride causes Ferric chloride in the presence of polyphenol derivatives the appearance of a blackish green or blue coloring more or less dark. The control was performed with the alcoholic solution of gallic acid.

2.5. Antioxidant activity
The antioxidant activity was determined spectrophotometrically according to the method of Brand- Williams *et al.* [15] using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a free radical. A solution of 0.1 mM DPPH and Trolox standard solutions were prepared at different concentrations (1 / 1.5 / 2/3/4 mM) in methanol. 0.1 ml of Trolox / MeOH solutions were mixed with 10 ml of the solution of DPPH / MeOH and then the absorbance of the solutions was measured after 30 minutes at 517 nm. 0.1 ml of the extract was mixed with 10 mL of the solution of DPPH / MeOH and then the absorbance of this solution was measured after 30 minutes at 517 nm. The antioxidant activity is given in mmol Trolox equivalent (TEAC) / 100 g dry matter.

The results are expressed as antiradical activity or inhibition of free radicals as a percentage (% I) using the following formula [16-18]:

I% = [1- (Sample Abs - Abs negative control)] x 100

Where: I%: percentage of anti-radical activity (AAR%)
Abs Sample: Sample Absorbance;
Abs negative control: Absorbance of negative control.

2.5. Antibacterial activity

2.5.1. Preparation of plant materials

Leaves were air-dried on the herbarium table (25 - 28˚C) after which they were shredded and preserved in airtight cellophane bags. The shredded leaves were milled into powder form using a warring commercial blender. Fifty grams of each of the powdered plant materials was soaked in 100 mL of sterile distillated water. The flasks were manually agitated at intervals for three days. The resulting extract from each flask was filtered rapidly through a filter to 0.2 µm of diameter impermeable to fungi. [19-20]

2.5.3. Organisms test

Staphylococcus aureus and *Escherichia coli* strains were obtained from the National Hospital Center (NHC), Nouakchott, Mauritania. Isolates were from clinical sample of two patients suffering from pyogene dermatologic and urinal infections, respectively. The isolates identities were further confirmed in our laboratory using standard biochemical procedures (Barrow and Feltham, 1993) [21]. The isolates were maintained on Tryptone Soy Agar (TSA) at 4C before use for this work.

2.5.4. Determination of antibacterial activity

The medium used was Mueller Hinton agar [22] Overnight cultures (0.2 ml) of each test microorganism was dispensed into 20 mL sterile nutrient broth and incubated for 4 h to standardize the culture to 10^6 cfu /ml (Collins et al. 1995) [23]. The bacterial inoculums were inoculated onto the medium using sterile swabs. For each extract, three replicate plates were prepared against the test organisms. Antibacterial activity of extracts of the plant samples was evaluated by the agar disk diffusion method using sterile disks buvard paper of 6 mm of diameter equidistant of the agar plats. 1 mL of each bacterial suspension was ensemenced with innodation, eliminer l excess, incubated the plats at 37 C for 15 mn. The disk impregnated with each extract was deposed in the plat. The plates were left for 2 h at room temperature to allow the extract to diffuse. In the each plate, sterile distillated water was used for extraction served as negative control. The antibiotic Cefotaxime (CTX) disk (30 µg/ml) was used as positive control for comparison. The plate was then incubated at 37 C for 18 h. Antibacterial activity was determined by measurement of zone of inhibition around each disk using a pair of calipers (in mm) and read on a meter rule.

RESULTS AND DISCUSSION

3.1. Ethnobotanical study

The survey was conducted in the city of Nouakchott, capital of Mauritania, and respondents are from ethnic and different locations across Mauritania. The majority of respondents are men aged 50 to 70 years and various professions (traditional medicine, merchants, maids, etc.).

We interviewed 23 people and the results obtained are summarized in Figures 1-4.

The results show that the leaves of *Tapinanthus pentagonia* are used as remedies for various pathologies: for example, against stomach ache, diarrhoea, tuberculosis, in addition to their veterinary uses.

Figure 1 : veterinary use

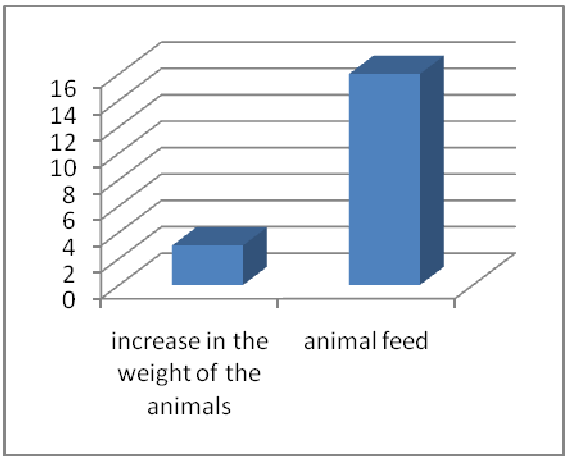


Figure 2: Frequency of Use

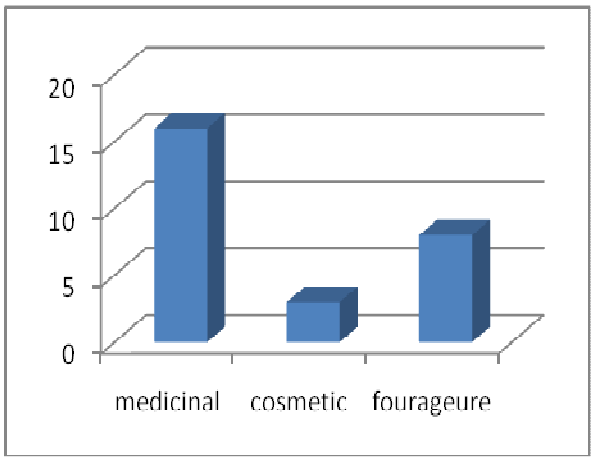


Figure 4: Frequency of symptoms treated with the Tapinanthus pentagonia

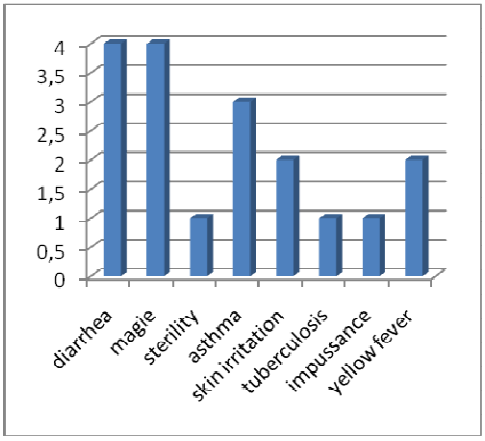
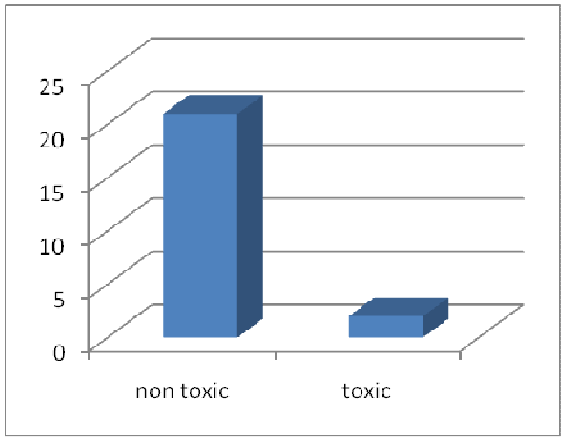


Figure 3: Distribution of respondents according to their opinion



3.2. Phytochemical screening

The results of phytochemical tests are summarized in Table I. This table shows that the fruits of *Tanpinanthus pentagonia* contain, flavonoids, ellagic acid, tannins, polyphenols, saponins reducing sugar, proanthocyanidins, sterols and terpenes. However, this study shows that quinones and alkaloids were not present.

Table 1: Different groups of compounds identified in *Tapinanthus pentagonia*

phytochemical compounds	Reaction / reagents	Observations
Tannins	SR ,HCl	+
Saponins	MT	+
Polyphenols	$FeCl_3$ à 2%	+
Proanthocyanidols	HCl	+
Ellagic acid	Acetic Acid	+
Flavonoids	RC	+
Sterols and terpenes	LR	+
Quinones	BGR	-
Alkaloids	DR , BR	-

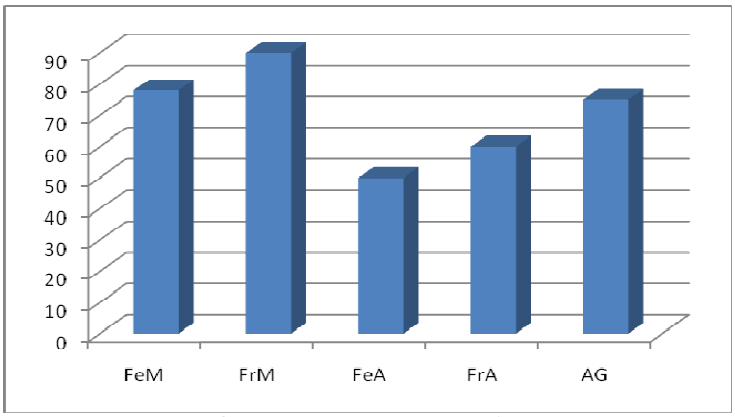
RS= Reagent Stiasny; MT= Mousse test; RC= Reaction to Cyanidin; LR= Liebermann reaction; BGR = Borntraeger reagent; DR= Dragendorff reagent; BR= Burchard reagent

3.3. Measurement of antioxidant activity

The results are expressed as a percentage of the anti-radical activity (Figure5) Evaluation of antioxidant activity by DPPH test revealed a great antioxidant for all extracts, methanol extract has a powerful antioxidant effect with 90.65% and 77.57% values of fruits and leaves, respectively, while the aqueous extracts have a lower activity than methanol extracts 60.19% and 50.65% of flowers and leaves. The results also show that the activity of extracts of the flowers is stronger than that of the leaves as explained by the wealth of flowers in polyphenols

For comparative purposes, gallic acid is used as a reference; it shows a powerful antioxidant activity 75.5%.

Figure 5 : Percentage inhibiting DPPH



GEF = methanol sheet; Frm = Flower Méthnolique; FEA = Aqueous Leaf; FrA = Aqueous Flower; AG = Gallic Acid

3.4. Antibacterial activity

The results of the antibacterial activities of the water extracts show that the two plants demonstrated activities against *S. aureus* to varying degrees. The two extracts Tpr and Tzm showed no activity against *E. coli*. The results obtained suggested that the host plants on which *T. pentagonia* are found can influence the antibacterial activity of the plant,in good agreement with those obtained for other related species such as *T. pangwensis*, but with methanol and chloroform extracts (Efuntoye MO et al 2010). All *Loranthaceans* are parasites of the xylem tissue and depend on their hosts for water, nutrients and some carbon compounds (Didier et al. 2008). This may be added to their antibacterial activity since many of the plants on which they parasitize are known to have medicinal properties, and hence there may have been nutritional exchange with the host tissues.

The aerial parts of this plant are widely used in African traditional medicine for treating different types of respiratory diseases and others. The study reveals the antimicrobial activity of *T pentagonia* against *S aureus* isolated from a dermatologic infection. This activity seems to be influenced by the nature of the plant host. Such a study should be extended to a larger number of plants and in different regions in Mauritania.

Table II . Antibacterial activity of *Tapinanthus pentagonia* against selected bacteria

		Zone of inhibition (mm)		
Test organism	Plant Tpr	Plant Tzm	Temoin positif CTX disk	Temoin negatif
E. coli	0	0	34	0
S. aureus	13	24	35	0

Tpr*: Tapinanthus pentagonia* on *Piliestigma retimlatirum*,
Tzm: *Tapinanthus pentagonia* on *Ziziphus mauritania*

CONCLUSION

Our own ethnobotanical surveys at traditional therapists in Mauritania show that the fruits and leaves of *Tanpinanthus pentagonia* are used, among others purposes, against jaundice, kidney stones since they are considered stomachic, diuretic and liver tonics. The phytochemical study showed the presence of secondary metabolites that have a potential economic and medicinal interest. Finally, the study of antioxidant activity revealed the presence of an activity for methanolic and aqueous extracts of *Tanpinanthus pentagonia* fruit and leaves. Further research is underway to isolate and identify these secondary metabolisms.

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