



Antibacterial and Antifungal Properties of Some Wild Nutraceutical Plant Species from Nebbi District, Uganda

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Authors' contributions

This work was carried out in close collaboration between all authors. Author GA wrote the original concept, study design, managed the literature searches and conducted the field and laboratory work. Authors HOO and MKM were involved monitoring advising and guiding the progression of the study, proof reading and editing the manuscripts. All authors participated in writing the final manuscript and have read and approved it.

Original Research Article

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ABSTRACT

Aims: The purpose of this study was to investigate the antibacterial and antifungal properties of selected wild nutraceutical plants from Nebbi district in Uganda.

Study Design: Experimental study.

Place and Duration of Study: The study was carried out at the Department of Veterinary Microbiology and Parasitology, College of Veterinary Medicine, Animal Resources and BioSecurity, Makerere University, between January and March 2012.

Methodology: The diameters of the zones of inhibition and the Minimum Inhibitory Concentrations (MIC) were determined using the Agar well diffusion Assay and the serial dilution methods respectively.

Results: Seven plant species were tested for their antibacterial and antifungal activity against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Candida albicans* (clinical isolates). The ether extract for *Balanites aegyptiaca* (L.) Delile showed the lowest MIC (150 µg/ml) against *C. albicans*, with a corresponding large diameter of the zone of inhibition (22.0

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mm). The ether extract of *Capparis erythrocarpos* Isert. showed the highest activity against *C. albicans* and *S. aureus*, with MIC values of 330 µg/ml and 400 µg/ml respectively.

Conclusion: *Balanites aegyptiaca* (L.) Delile, and *Capparis erythrocarpos* Isert were the most potent antifungal and antibacterial nutraceutical plant species. The ether extract of *Balanites aegyptiaca* (L.) Delile, had the lowest MIC (150 µg/ml) against *C. albicans* and *Capparis erythrocarpos* Isert. showed the highest activity against *C. albicans*, *S. aureus*, and *E. coli* with MIC values of 330 µg/ml, 400 µg/ml and 320 µg/ml, respectively. The bioactivity of the selected wild nutraceutical plant species can be used to justify their ethnobotanical uses as 'medicinal foods'.

Keywords: Antibacterial; antifungal; MIC; wild; nutraceuticals; Nebbi.

1. INTRODUCTION

Wild plants grow spontaneously in self-maintaining populations in natural or semi-natural ecosystems and can exist without purposeful cultivation [1,2,3]. The majority of foods globally are neglected or underutilized and are generally wild [4,5]. They provide a greater dietary diversity and have a comparable or richer nutritional composition than cultivated ones [6,7]. Widespread empirical use of wild plants demands accurate and reliable information on their phytochemicals and the potential benefits and prospective products, such as nutraceuticals and phytomedicines [8].

Many wild food plants are also used for medicinal purposes [9,2,10]. Hippocrates, 400–377 B.C. is known to have said 'Let food be your medicine and medicine be your food' [11]. There has been renewed interest in consuming wild food plants [12,13] as they have specific pharmacological effects [14,15]. Furthermore, they help to reduce risk from a variety of chronic and inflammatory conditions, certain types of cancers, diabetes, menopausal symptoms and age related diseases and are also beneficial to households afflicted by HIV/AIDS among others [16,11,6,17].

Nutraceuticals are a combination of nutritional and pharmaceutical compounds that act as medicines [18,19]. They are becoming more widely accepted as an adjunct to conventional therapies [16]. Many of these "food-medicines" are beneficial beyond their nutritional value because they contain a variety of plant secondary compounds such as anthocyanins, tannins, carotenoids, flavonoids, phenols and antioxidants among others [17]. These phytochemicals are also called antinutritional factors and are generally not essential for normal functioning of the body but have important therapeutic functions [16,20,14].

In Africa, many wild food plants remain untapped and are frequently neglected by researchers and policy makers [21,4,22]. They are considered to be of low-status and only appropriate for the poor [21,22]. A good number of medicinal plants used by local communities in Uganda may have potential for wider applications not yet known to man [23] and many more wild species are believed to be edible but not yet documented [13]. The purpose of this study therefore was to investigate the antibacterial and antifungal properties of some wild nutraceutical plants from Nebbi district in Uganda.

2. MATERIALS AND METHODS

2.1 Study Area

Nebbi District is located in the North-Western part of Uganda, in the equatorial forest belt extending from Democratic Republic of Congo (DRC). It occupies a total area of 3,288 km² and lies between 2° 44' N; 31° 24' E at an altitudinal range of 945–1, 219 m above sea level [24]. Nebbi has an average rainfall of 1,500 mm and high temperatures of the modified equatorial climatic type [24]. Plant specimens and samples were collected from Panyango Sub County in Nebbi district.

2.2 Collection of Voucher Specimens

In a follow up to an earlier ethnobotanical study by Anywar et al. [22], seven plant species were selected for analysis, basing on their frequency of mention, multiplicity of uses especially as food and medicine and conservation status. These plant species were *Balanites aegyptiaca* (L.) Delile, *Capparis erythrocarpos* Isert., *Leptadenia astate* Vatke, *Nymphaea lotus* L., *Sclerocarya birrea* (A. Rich.) Hochst., *Senna obtusifolia* (L.) H.S.Irwin & Barneby and *Talinum portulacifolium* (Forsk) Asch.ex Schweinf. Voucher specimens of each of the plant species were collected, processed according to standard procedures described by Martin [25], and taken to the Makerere University Herbarium for identification and classification, basing on the database at <http://www.theplantlist.org> version 1.1 accessed on 1st May 2014.

2.3 Preparation of the Plant Extracts

Samples of each of the seven plant species were collected from the field, dried in the laboratory for two weeks and stored in an air-tight containers, prior to the analyses. The dried plant samples (250 mg) of each plant species were soaked in 500 ml of petroleum ether for four days. The samples were then filtered and the residue was air-dried for two days. After drying, the same plant material was soaked in 500 ml methanol for four days. The extract (250g) of each sample was separately dissolved in two drops of 10% dimethylsulphoxide (DMSO) and 10 ml of distilled water to obtain a stock solution of 250 mg/ml which were kept at 4°C prior to the assay.

2.4 Preparation of the Medium

Meuller-Hinton agar powder (40 g) was added to 1 litre of distilled water and the mixture was boiled. The solution was autoclaved at 121°C for 15 minutes at 15 psi and cooled to 50°C in a water bath then transferred into sterile Petri dishes. It was allowed to cool further and solidify under sterile conditions and then incubated for 24 hours at 37°C.

2.5 Preparation of Test Organisms

Extracts from the plant samples of the different species were tested against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Candida albicans* (clinical isolates). They were obtained from the Department of Microbiology, College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, and incubated for 18 hours, before use.

2.6 Antibacterial Screening (Agar-well Diffusion Assay)

Cultures of the test organisms were inoculated separately on the solidified agar on each Petri dish. Ciprofloxacin (50 mg/ml) and Fluconazole (50 mg/ml) were used as positive controls for the bacteria and the fungus respectively. They were dispensed into the uniformly dug wells of 10 mm diameter. The wells were filled to about $\frac{3}{4}$ way and the plates were incubated at 37°C for 24 hours. Distilled water and DMSO were used as negative controls. The sensitivity of the test organisms to the plant extracts were determined by measuring the diameters of the zone of inhibition (Table 1).

2.7 Determination of Minimum Inhibitory Concentration (MIC)

The MIC values were determined by serial dilution method (Table 2). Two dilutions of the stock plant extract solution in bacterial broth were prepared. Two test tubes were arranged in a row and serial dilutions of the crude plant extracts were carried out with 250 mg/ml as the highest concentration in tube. Distilled water (0.5 ml) was poured in each test tube, and then 0.5 ml from tube 1 was poured in tube 2. It was mixed well and the process repeated.

3. RESULTS

The ether extract of *Capparis erythrocarpos* Isert. showed the highest activity against *C. albicans* (26.3 mm) and against *S. aureus* (26.3 mm) (Table 1). It also showed high activity against *E. coli*. However, the methanol extracts of *Capparis erythrocarpos* Isert., did not have any activity *E. coli* and *P. aeruginosa*. The methanol extract of *Sclerocarya birrea* (A. Rich.) Hochst. showed the highest activity (25.3 mm) of all the methanol extracts against *S. aureus*. The same extract also showed high activity against *E. coli*.

Only *Nymphaea lotus* L. and *Sclerocarya birrea* (A. Rich.) Hochst. Showed activity against all the 4 test organisms. These two plant species were also the only ones whose methanol extracts were active against *P. aeruginosa* (19.7 mm) and (15.0 mm) respectively. The ether extracts of *Capparis erythrocarpos* Isert., (13.0 mm), *Sclerocarya birrea* (A. Rich.) Hochst. (10.7 mm) *Leptadenia hastata* Vatke (7.3 mm), and *Nymphaea lotus* L. (7.0 mm) were the only ones active against *P. aeruginosa*. All the methanol extracts of *Talinum portulacifolium* (Forsk) Asch.ex Schweinf. and *Leptadenia hastata* Vatke were inactive against all the test organisms.

A classification based on MIC values proposed by Algiannis [26], was used for this study. Extracts with MIC values up to 500µg/ml were considered strong inhibitors, 600–1500µg/ml as moderate inhibitors and those above 1600 as weak inhibitors. The ether extract for *Balanites aegyptiaca* (L.) Delile, showed the lowest MIC (150 µg/ml) against *C. albicans* (Table 2). These were followed by the MIC values of the methanol extract of the bark of *Sclerocarya birrea* (A. Rich.) Hochst. against *E. coli* and the ether extract of the same tree species against *S. aureus* (300 µg/ml). Some plant extracts had high MIC values for example *Talinum portulacifolium* (Forsk) Asch.ex Schweinf., *Senna obtusifolia* (L.) H.S.Irwin & Barneby and *Capparis erythrocarpos* Isert. and were therefore considered weak inhibitors while others like the ether and methanol extracts of *Talinum portulacifolium* (Forsk) Asch.ex Schweinf. were not active at all against *P. aeruginosa*.

Table 1. Diameters of zones of inhibition of plant extracts on the test organisms (mm)

Plant name	PU	<i>C. albicans</i>		<i>E. coli</i>		<i>S. aureus</i>		<i>P. aeruginosa</i>	
		E	M	E	M	E	M	E	M
1. <i>Balanites aegyptiaca</i> (L.) Delile	B	0.0±0.00	22.0±0.58	16.0±1.00	0.0±0.00	22.0±1.00	0.0±0.00	0.0±0.00	0.0±0.00
2. <i>Capparis erythrocarpos</i> Isert.	L	26.3±2.03	10.7±0.58	25.3±1.53	0.0±0.00	26.3±0.58	10.3±0.58	13.0±0.00	0.0±1.00
3. <i>Leptadenia hastata</i> Vatke	L	19.3±1.15	0.0±0.00	17.3±1.53	0.0±0.00	15.7±0.58	0.0±0.00	7.3±0.58	0.0±0.00
4. <i>Nymphaea lotus</i> L.	Sd	11.7±1.53	19.3±0.58	10.0±1.00	19.0±1.00	12.3±0.58	15.7±0.58	7.0±0.58	19.7±0.58
5. <i>Sclerocarya birrea</i> (A. Rich.) Hochst.	B	17.7±1.53	21.0±1.00	15.3±0.58	25.3±1.53	22.7±1.15	25.3±0.58	10.7±0.58	15.0±1.00
6. <i>Senna obtusifolia</i> (L.) H.S.Irwin & Barneby	L	11.0±0.00	0.0±0.00	9.3±0.58	10.7±0.41	11.7±0.58	12.7±0.58	0.0±0.00	0.0±0.00
7. <i>Talinum portulacifolium</i> (Forsk) Asch.ex Schweinf.	L	10.7±0.58	0.0±0.00	7.3±0.58	0.0±0.00	11.3±0.58	0.0±0.00	0.0±0.00	0.0±0.00

Values are means ± standard deviation (SD), n=7.

Key: PU=Part Used, E=Ether extract, M=Methanol extract, B=Bark, L=Leaves, Sd=Seeds

Table 2. Minimum inhibitory concentrations (MIC µg/ml)

Plant Name	PU	<i>C. albicans</i>		<i>E. coli</i>		<i>S. aureus</i>		<i>P. aeruginosa</i>	
		E	M	E	M	E	M	E	M
1. <i>Balanites aegyptiaca</i> (L.) Delile,	B	na	150	380	Na	470	Na	na	na
2. <i>Capparis erythrocarpos</i> Isert.	L	330	500	320	*	400	530	500	*
3. <i>Leptadenia hastata</i> Vatke	L	400	na	700	*	500	*	580	na
4. <i>Nymphaea lotus</i> L.	Sd	520	350	520	800	520	800	580	380
5. <i>Sclerocarya birrea</i> (A. Rich.) Hochst.	B	350	320	350	300	300	350	500	550
6. <i>Senna obtusifolia</i> (L.) H.S.Irwin & Barneby	L	*	na	*	*	*	*	na	na
7. <i>Talinum portulacifolium</i> (Forsk) Asch.ex Schweinf.	L	*	*	na	*	*	na	na	na

Key: PU=Part Used, E=Ether extract, M=Methanol extract, B=Bark, L=Leaves, Sd=Seeds, * =MIC > 1600 µg/ml, na =Not active

4. DISCUSSION

The high bioactivity of the ether extract of *Balanites aegyptiaca* (L.) Delile, showed by its low MIC (150 µg/ml) against *C. albicans* is in agreement with the findings of Maregesi et al. [27], who also reported *Balanites aegyptiaca* (L.) Delile, stem bark to have exhibited the highest antifungal activity against *C. albicans* MIC (125µg/ml). These were followed by the MIC values of the methanol extract of the bark of *Sclerocarya birrea* (A. Rich.) Hochst. against *E. coli* and the ether extract of the same tree species against *S. aureus* (300 µg/ml). The bark of *Sclerocarya birrea* (A. Rich.) Hochst. has also been shown to be active against *E. coli*, *S. aureus* and *P. aeruginosa* with values ranging from 0.15-3 mg/ml, depending on whether it is the inner bark or outer bark used [28]. The bark of *Sclerocarya birrea* (A. Rich.) Hochst. has also been shown to have strong antifungal activity against *C. albicans* [29]. The leaves and seeds of *Sclerocarya birrea* (A. Rich.) Hochst. are eaten as sauce in Nebbi, while an infusion from the bark is used for treating stomach ache, haemorrhoid's, prolapsed rectum and astate [22].

Although there were some similarities in the MIC values from this study with those of other studies, some differences are also noted. For example the MIC values of *Balanites aegyptiaca* (L.) Delile, reported in this study were higher than those reported by Maregesi et al. [27]. Such discrepancies in findings may be due to the use of different pathogen strains, differences due to the extraction process and solvent used. For example, clinical isolates of *C. albicans* were used for this study while Maregesi et al. [27], used *C. albicans* (ATCC 10231) strain for their analyses. The fruit of *Balanites aegyptiaca* (L.) Delile, are eaten as snack and cooking oil extracted from seeds. A bark decoction is used to treat joint aches, stomach pain and fever [22].

The ether extract of *Capparis erythrocarpos* Isert. showed the highest activity against *C. albicans* and *S. aureus*. *Capparis erythrocarpos* Isert. has been reported to be used in treating patients with HIV/AIDS, tonsillitis, diarrhea, cataracts and eaten as a vegetable in Uganda [30,31,22]. Most of the plant species had high MIC values indicating mild to weak antibacterial and antifungal activity. Despite the weak bioactivity shown by some of the plant species, it should be put into consideration that most of the plant species are consumed as food. Most of the plant species tested thus showed weak to moderate activity against one or more of the microorganisms.

The tender leaves and flowers of *Leptadenia hastata* Vatke are eaten as sauce, but also used to treat flu, wounds, cough, fever and body weakness [22]. The seeds of *Nymphaea lotus* L. are eaten as porridge or bread during famines [22]. The leaves of *Senna obtusifolia* (L.) H.S.Irwin & Barneby are eaten as vegetable and warm water infusion of leaves used to treat stomach aches [22]. The leaves of *O. sinuatum* eaten as sauce and used to treat skin infections, oral and anal sores in Nebbi district [22], and skin and throat cancer in Kenya by [32]. Leaves of *Talinum portulacifolium* (Forsk) Asch.ex Schweinf. are cooked and eaten as a vegetable and infusion used to treat stomach ache in Nebbi [22]. The leaves have been reported to treat malaria in Budiope County, eastern Uganda [33], gastritis in Somalia [34]. The leaves are also eaten raw as a salad with young stems and also used to treat diabetes in developing countries [35].

5. CONCLUSION

Balanites aegyptiaca (L.) Delile and *Capparis erythrocarpos* Isert were the most potent antifungal and antibacterial nutraceutical plant species. The ether extract of *Balanites aegyptiaca* (L.) Delile, had the lowest MIC (150 µg/ml) against *C. albicans* and *Capparis erythrocarpos* Isert. Showed the highest activity against *C. albicans*, *S. aureus* and *E. coli* with MIC values of 330 µg/ml, 400 µg/ml and 320 µg/ml, respectively. The bioactivity of the selected wild nutraceutical plant species can be used to justify their ethnobotanical uses as 'medicinal foods' in the study area.

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CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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