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Assessment of the Antimicrobial and Antioxidant Activities and Total Phenolic Content of Crude Extracts from *Boscia Senegalensis* Sudan

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ABSTRACT

The objective of the present study was the assessment of the antimicrobial and antioxidant activities of Boscia senegalensis., from n-hexane, chloroform, ethyl acetate and methanol extracts. In addition, the total polyphenolic, flavonoids and tannins contents of these extracts were determined and were screened for their antimicrobial activity using cup diffusion assay against four standard strains of bacteria, two Gram-positive strains (Bacillus subtilis and Staphylococcus aureus), two Gram-negative bacterial strains (Escherichia coli and Pseudomonas aeruginosa) and two fungal strains (Aspergillus niger and Candida albicans). Antioxidant activity was screened using 2,2-Di(4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH). Total polyphenolic, flavonoids and tannins contents were determined by spectrophotometric assays. Generally, the results of antimicrobial activity showed that extracts of the plant exhibited better antifungal activity than antibacterial. The highest antifungal activity against A.niger and Candida albicans., was recorded from the methanolic extract and ethyl acetate of Boscia senegalensis., (inhibition zone (IZ) 16±0.58 and 14±0.58mm respectively), flowed by methanolic extract against C.albicans., (IZ =12±0.58mm); while the highest antibacteria activity of the n-hexane extract against Staphylococcus aureus, and E.coli., gave the best activity (IZ = 11±2.51 and 11±1.52mm). The highest scavenging radical activity was obtained from the methanol extract and gave (17±0.02%). Aquantitative analysis revealed the total polyphenolic value, with the highest result shown in ethyl acetate (71.12±0.06mg gallic acid equivalent (GAE)/g) and the flowed chloroform extract (63.19±0.19mgGAE)/g). The highest total flavonoids content value was recorded from the chloroform extract (411.26±0.06mg quercetin equivalent/g), flowed by ethyl acetate extract (329.49±0.04mg quercetin equivalent/g), while the highest total tannins content value was found in methanol extract (189.44±0.05mg tannic acid equivalents/g) flowed n-hexane extract (101.21±0.13mg). In conclusion, the studied plant was rich in bioactive agents with antioxidant and antimicrobial potential and could have interesting pharmaceutical and cosmetic applications. Determination of the bioactive compounds from the active plant extracts and their mode of action are recommended.

Keywords
Boscia senegalensis; antimicrobial activity; Antioxidant activity; Total phenolic; Sudan.

1. INTRODUCTION

Medicinal plants are plants which contain substances that could be used for therapeutic purposes, or which are precursors for the system of useful drugs (Mohamed and Mustafa, 2019). *Boscia senegalensis.,* Pubescent shrubs or small trees, up to 3 m high, leaves alternate, oplong elliptic to obovate, 3.8-10.0 x1.7- 4.9 cm, apex apiculate or mucronate, base cuneate, margin entire, inflorescences dense corymbs, peduncles up to 3cm long, pediceks 2-3cm long, gynophores 2-4 mm long. Fruit drupes, globose, warted, up to 1.3cm long, pale yellow, 1 seed (Elghazali *et al.,*1994).

The leaves of *B. senegalensis* are traditionally used for human and animal nutrition, Protection of cereals against pathogens, and pharmacologic purposes (Salih *et al.,*1991). In Sudan, Kordofan province leave infusion is widely used medication for ventral disease, inflamed eye is also bathed with it. Root prepared are used for Jaundice root, leave and bark are used as coagulating agent to clarify water. In west of Sudan and Blue Nile *B. senegalensis* considered as one of the potent plants for treated, turbid water. Water coagulation is believed to protect from disease like; Diarrhea, gastro-intestile disorder, gastric fever (Awa *et al.,* 2020). Whole plant use as anthelimentic were as the emulsion of leaves used as an eye wash (Elgazali *et al.,* 1997).

B. *senegalensis.,* possesses antimicrobial and anti fungal activities, it has been shown to be effective uterine stimulation (Almagboul *et al.,* 1988). The leave used in the preparation of

malaria remedy and for the treatment of jaundice, fungal infection and viral diseases, it applied externally for wound. The fruits and roots are used as aphrodisiac, and the roots decoction is used for stomachache and to facilitate labour (Maurice,1992).

2. Materials and Methods

2.1 Plant Material:

The leaves of *Boscia senegalensis.*, was collected in March 2023 from Obeid, North Kordofan State, Sudan. The plant species was taxonomically identified by Dr. Mubarak Siddig Hamad, herbarium Department of Taxonomy and Phytochemistry, Medicinal, Aromatic and Tradition Medicine Research Institute, National Center for Research, Khartoum, Sudan. The plant was washed thoroughly under running water to remove contamination and was shade dried with active ventilation at ambient temperature for 5 days; the dried aerial parts were to fine powder using pistil and mortar.

2.2 Preparation of extracts

Separately, 20g of dried powdered leaves of *Boscia senegalensis.*, was extracted consecutively by maceration in hexane, chloroform, ethyl acetate and methanol (400 mL each) using a shaker apparatus, for about 24 h at room temperature, filtered and then solvents were evaporated under vacuum using a rotary evaporator. The resultant dry extracts from each sample was weighted and stored at 4°C until used.

2.3 Antimicrobial activity

The bacterial cultures used were *Bacillus subtilis* NCTC 8236, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 10145. The used fungi cultures were *Aspergillus niger* ATCC 9763 and *Candida albicans* ATCC 7596. Each extract (10 mg/disc) was tested using the disc diffusion method as described (Mbavenge and Coworkers, 2008). 20 µg from each extract was then used to impregnate a blank sterilized disc and were left to dry. A bacteria culture (which has been adjusted to 0.5 McFarland standard), was used to lawn Muller Hinton agar plates evenly using a sterile swab. Sabouraud medium was used for fungi. The impregnated discs were placed on the surface of dried plates. The standards: gentamicin and nystatin at a concentration of 10 mcg/disc served as the positive control for evaluation of the antibacterial and antifungal activities, respectively and DMSO (100%) as the negative control. Plates were then incubated at 37° C for 24 h for bacteria and at 25° C for 2-3 days for the fungi. Results were documented by measuring the zone of inhibition in mm.

2.4 Antioxidant activity

The antioxidant activity of the extracts was evaluated using the *in vitro* 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method (Grochowski *et al.,* 2017). The reaction mixture consisted of 1.0 ml of DPPH in methanol (0.3 mM) and 1.0 ml of the extract (1.56 - 50 µg/ml). Thereafter, it was incubated in the dark for 10 min, after which the absorbance was measured at 517nm. Propyl gallate (1.56-50 µg/mL) was used as a positive control.

2.5 Quantitative determination of total polyphenol, flavonoids and tannins contents

2.5.1 Determination of total polyphenols content

The total polyphenolic content was determined by adopting the method described (Wolfe *et al.,* 2003). The extract (1 mg/ml) was taken in a 10 ml glass tube and made up to a volume of 3 ml with distilled water. 0.5 ml Folin ciocalteau reagent (1:1 with water) and 4 ml sodium carbonate (7.5%) were added sequentially in each tube. The test solution kept in dark for 30 min, cooled and absorbance was measured at 765 nm. The total phenolic contents were expressed as mg gallic acid equivalents (GAE) /g) using the following equation based on the calibration curve: (Y=0.005X+0.000) where x = concentration of gallic acid (100 - 900 mg/g) as standard.

2.5.2 Determination of total flavonoids content

The total flavonoid content was determined by adopting the method described (Ordonez *et al.,*2006). Aliquots of each extracts were pipette out in series of test tubes and volume was made up to 2 ml with distilled water, 0.3 ml of sodium nitrite (5%) was added to each tube and incubated for 5 min. at room temperature, 0.3 ml of aluminum chloride solution (10%) was added and incubated for 5 min, 2 ml of sodium hydroxide (1M) were added. Absorbance was measured at 415 nm against a reagent blank. Total flavonoids content was expressed as mg quercetin equivalent (QE)/g) using the following equation based on the calibration curve: (Y=0.0012X+0.0958) where x = concentration of quercetin (100–900 mg/g) as standard.

2.5.3 Determination of total tannins content

Total tannins content was determined according to the procedure reported (Sun *et al*., 1998). A volume of 1 ml solution was mixed with 3 ml of 4% vanillin/methanol solution and 1.5 ml hydrochloric acid and the mixture was allowed to stand for 15 min at room temperature. The absorbance at 500 nm was measured and the tannins content was expressed as mg tannic acid equivalents (mg TAE/g dry mass) using calibration curve:(Y=0.002X+0.591) where x = concentration of tannic acid (100–900 mg/g) as standard.

2.6 Statistical analysis

All the procedures for extraction, antimicrobial, antioxidant activity and total phenolic content studies were repeated in triplicate. The descriptive analysis (mean and standard deviation) was used to discuss the results, assuming the normal distribution of the studied variables.

3. Results and Discussion

3.1 Antimicrobial activity

Hexane, chloroform, ethyl acetate and methanol extracts of *Boscia senegalensis.*, was evaluated for their antimicrobial activity. Results are depicted in Table1. Inhibition zone value <14 mm is considered as resistance, 14-18 mm is intermediate and >18 mm is sensitive (Arbonnier, 2002). Extracts from the studied plant displayed variable antimicrobial activity. The highest antifungal activity against *A.niger* and *Candida albicans.,* was recorded from the methanolic extract and ethyl acetate of *Boscia senegalensis.*, (inhibition zone (IZ) 16±0.58 and 14±0.58mm respectively), flowed by methanolic extract against *C.albicans.,* (IZ =12±0.58mm); while the highest antibacteria activity of the n-hexane extract against *Staphylococcus aureus,* and *E.coli.,* gave the best activity (IZ = 11±2.51 and 11±1.52mm). Variation in results from different methods could be attribute to to different factors like genetics, ages of the plant and environmental condition an any others (Dinnage *et al.,* 2019).

Table 1: Antimicrobial activity of crude extracts of *Boscia senegalensis.*

Botanical name	Organ	Extract	Inhibition zones diameter (IZD) in mm					
			B. s	*S. a*	*E. c*	*P. a*	*A. n*	*C. a*
B. senegalensis	Leaves	n-hexane	10±0.58	11±2.51	11±1.52	NA	10±1.00	9±5.51
		chloroform	NA	10±0.58	NA	NA	10±0.58	9±0.58
		Ethyl acetate	NA	NA	NA	NA	11±0.00	14±0.58
		Methanol	10±0.58	9±5.51	NA	NA	16±0.58	12±0.58
Gentamicin*		10µg/disc	15±0.50	13±0.00	17±0.10	14±0.58	NA	NA
Nystatin*		10µg/disc	NA	NA	NA	NA	22±0.01	20±0.05

NA: not active, * positive control (10µg/disc) *B*.s = *Bacillus subtiles.* S.a = *Staphylococcus aureus,* E.c = *Escherichia coli*, P.a = *Pseudomonas aeruginosa*, A.n = *Aspergillus niger*, C.a = *Candida albicans.* IZD (mm): > 18mm: Sensitive: 14-18mm: intermediate: < 14mm: Resistant.

3.2 Antioxidant activity

Antioxidant activity of extracts from the plant was determined by evaluating their capacity to scavenge the DPPH free radicals and results are presented in Table 2. The highest scavenging radical activity was the methanol extract gave highest activity (17±0.02%), while the ethyl acetate extract non active (Table 2).

The results of the present study indicate the nutritional and functional potential of the wild *B.senegalensis.,* from Obeid, since it shows contents of compounds with free radical scavenging capacity equivalent to those of several edible plants or medicinal use, which reinforces its potential for consumption. It is important to note that the variation found in accessions could be attributed to climatic and growth conditions; however, more in depth studies are needed to designate which parameters have the most influence on these variations.

Table 2: Antioxidant activity of *B.senegalensis.,* :

Plant name	Organ	Extract	%RSA±SD (DPPH)
B.senegalensis	leaves	N-hexane	9±0.04
		Chloroform	6±0.03
		Ethyl acetate	In active
		Methanol	17±0.02
Standard	SD	Propyl gallate	94±0.01

RSA=Radicals scavenging; DPPH= 2,2, Diphenyl-1 Picrylhydrazzyl.

3.3 Total polyphenolic, flavonoids and tannins contents

Results of total polyphenolic, flavonoids and tannins contents of different extracts from the plant is presented in Table3. Phenolic compounds are known as powerful chain breaking antioxidant (Al-Snafi, 2015) and they arevery important plant constituents because of their scavenging ability, which is due to their hydroxyl group (Bendary, *et al.,* 2013). Aquantitative analysis revealed the total polyphenolic value, with the highest result shown in ethyl acetate (71.12±0.06mg gallic acid equivalent (GAE)/g) and the flowed chloroform extract (63.19±0.19mgGAE)/g).

The highest total flavonoids content value was recorded from the chloroform extract (411.26±0.06mg quercetin equivalent/g), flowed by ethyl acetate extract (329.49±0.04mg quercetin equivalent/g). While the highest total tannins content value was found in methanol extract (189.44±0.05mg tannic acid equivalents/g), flowed n-hexane extract (101.21±0.13mg). Variation in polyphenolic and falvonoids contents of the studied species from values reported for the same studied species in the literature could be attributed to different factors like geographical areas and climatic conditions for the growth of the plant(Khurm, *et al.*, 2020).

Botanical name/ studied part	Extract	Total phenol content (Y=0.005X+0.000) R^2= 0.998	Total flavonoids content (Y=0.0012X+0.0958) R^2=0.09915	Total tannin content (Y=0.002X+0.591) R^2=0.997
B. senegalensis	N-hexane	46.36±0.01	131.01±0.00	101.21±0.13
	chloroform	63.19±0.19	411.26±0.06	69.81±0.07
	Ethyl acetate	71.12±0.06	329.49±0.04	64.04±0.21
	Methanol	59.19±0.03	100.16±0.02	189.44±0.05

GAE: Gallic acid equivalent; QE: Quercitin equivalent; TAA: Tannic acid equivalent.

4. Conclusion

Extracts of different polarity from the plant showed variable antimicrobial and total phenolics. The inhibitory zones of different extracts varied with the type of microorganism tested. Generally, extracts of the plant exhibited better antifungal activity than antibacterial. The majority of extracts were rich in flavonoids, while the polyphenols were mainly accumulated in the two polar extracts. Therefore, this plant could a very beneficial source of natural bioactive agents. Further studies should be undertaken to elucidate the particular phytochemicals and their pharmacological mechanism.

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