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Antimicrobial, Antioxidant Activities and total phenolics contents of *Portulaca oleracea*., crude extracts, Sudan

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Abstract

Drug-drug interaction is a public health problem that constitutes one of the leading causes The objective of the present study was to evaluate the *in-vitro* antimicrobial, antioxidant, and total polyphenolic, flavonoids, and tannin contents of crude extracts of *Portulaca olerace*. Extracts from each plant were prepared by sequential maceration of dried whole plant powder in solvents of increasing polarity. The antimicrobial activity was evaluated against Gram-positive and Gram-negative, as well as two fungi, by the disc diffusion method. Antioxidant activity was assessed based on the scavenging activity of the stable 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH). Total polyphenolic, flavonoids, and tannin contents were determined by spectrophotometric assays. Generally, the results of antimicrobial activity showed that extracts of the plant exhibited better antifungal activity than antibacterial activity. The highest antifungal activity against *Candida albicans* was recorded from the methanolic extract of *P. olerace* (inhibition zone (IZ) =19mm) flowed by ethyl acetate extract (IZ =18mm). While the highest antibacterial activity of the methanolic extract against Pseudomonas aeruginosa was obtained from the disc diffusion method (IZ = 20 mm), The highest scavenging radical activity was obtained from the methanol extract (45%). Quantitative analysis revealed total polyphenols had the highest value shown in methanolic extract (114.17±0.15mg gallic acid equivalents/g). The total flavonoid content was the highest value recorded from the ethyl acetate extract (499.08±0.01 mg quercetin equivalents/g). While the total tannin content had the highest value found in methanol extract (532.18±0.13mg tannic acid equivalents/g).

KEY WORDS: *Portulaca oleracea*, antimicrobial activity, antioxidant activity, total phenolic, total flavonoids. etc.

1. INTRODUCTION

Sudan exhibits a wide range of variation in the topography, climate, soil, and hydrology; these result in different vegetation zones and consequently rich flora[1]. *Portulaca oleracea*. L. (Purslane), belonging to the family of Portulacaceae, was widely used in the Mediterranean, Central European, African, and Asian countries. It was listed by the World Health Organisation (WHO) as one of the most used medicinal plants and has been given the term 'Global Panacea'[2].

The aerial parts of the plant are used in many countries as a diuretic, febrifuge, antiseptic, antispasmodic, and vermifuge[3] and have a variety of pharmacological activities, including analgesic, anti-inflammatory, antifungal, antibacterial, wound healing, and hypoglycaemic[4]. *Portulaca oleracea* L. is a good source of beneficial compounds for human health, including omega-3, β-carotene, α-tocoferol, mucilages, and minerals[5]. Water was reported as the major constituent of purslane leaves and stems. Among the 27 fatty acids detected in the plant, the most abundant was linolenic acid, followed by palmitic and oleic acids. Citric, acotinic, fumaric, citric, malic, and oxalic acids were the organic acids that were found to exist in the plant[6,7]. This plant is considered a weed of almost worldwide distribution in Sudan, although in many regions it is appreciated for its nutritional and medicinal properties.

The antioxidant potential of *P. oleracea* has been considered a criterion for its consumption and use, so the exploration of accessions that have considerable levels of these compounds represents an opportunity to study the variations of these compounds in breeding plans[8,9]. Endogenously, they are

the main ones responsible for physiological and ecological functions, and it has been observed that their concentrations vary in situations of biotic and abiotic stress[10,11]. Therefore, the objective of this research was to determine the evaluation of the antimicrobial, antioxidant, and total phenolic contents of crude extracts of *P. oleracea* grown in Obeid, North Kordofan State, Sudan.

2. Materials and Methods

2.1 Plant Material

The aerial parts of *Portulaca olerace* were 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 Centre 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 ground to a fine powder using pistil and mortar.

2.2 Preparation of Extracts

Separately, 20 g of dried powdered aerial parts of *P. olerace* were 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 were weighted and stored at 4°C until used.

2.3 Preparation of Extracts

The bacterial cultures used were *Bacillus subtilis* (NCTC 8236), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 10145). The used fungus 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 by Mbavenge and coworkers[12]. 20 µg of each extract was then used to impregnate a blank sterilised disc and 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 the evaluation of the antibacterial and antifungal activities, respectively, and DMSO (100%) as the negative control. Plates were then incubated at 37°C for 24 hours for bacteria and at 25°C for 2–3 days for 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[13]. 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 minutes, 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 by Wolfe *et al.*[14]. 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 of Folin ciocalteau reagent (1:1 with water) and 4 ml of sodium carbonate (7.5%) were added sequentially in each tube. The test solution was kept in the dark for 30 minutes, 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[15]. Aliquots of each extract were pipetted out in a series of test tubes, and the 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 aluminium chloride solution (10%) was added and incubated for 5 min. 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 tannin content was determined according to the procedure reported[16]. A volume of 1 ml of solution was mixed with 3 ml of 4% vanillin/methanol solution and 1.5 ml of hydrochloric acid, and the mixture was allowed to stand for 15 minutes at room temperature. The absorbance at 500 nm was measured, and the tannin content was expressed as mg tannic acid equivalents (mg TAE/g dry mass) using the 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 *Portulaca olerace* were evaluated for their antimicrobial activity. Results are depicted in Table 1. Inhibition zone

value of 14 mm is considered as resistance, 14–18 mm is intermediate, and 18 mm is insensitive[17]. Extracts from the studied plant displayed variable antimicrobial activity. The highest antifungal activity showed that the methanol and ethyl acetate extracts against *C. albicans* gave inhibition zone (IZ) = (19±3.06 and 18±1.16mm respectively). While the highest antibacterial activity was recorded methanol and ethyl acetate extracts against *Pseudinhibition zonessa* (IZof=20±7.03 and 15±0.58 mm, respectively).

However, it is worth mentioning that this is the highest report on the antimicrobial activity of *P. olerace*. The methanolic extract of *Portulaca olerace* showed mild antimicrobial activity against the growth of *E. coli* (8mm), *Shigella bydii* (9 mm), *Pseudomonas aeruginosa* (9mm), and *Klebsiella pneumoniae* (8mm) when compared with the standard drug Amoxicillin (8–35 mm)[18]. Variation in results from different methods could be attributed to different factors like genetics, ages of the plant, environmental conditions, and any others[19].

Table 1: Antimicrobial activity of crude extracts of *Portulaca olerace*

Botanical name	Organ	Extract	Inhibition zones diameter (IZD)in mm					
			B.s	***S.a***	***E.c***	***P. a***	***A. n***	***C.a***
P.olerace	Aerial parts	n-hexane	9±5.81	NA	10±1.65	10±5.78	15±1.00	11±2.09
		Chloroform	NA	NA	NA	10±0.01	17±1.00	13±1.16
		Ethyl acetate	NA	NA	NA	15±0.58	12±6.66	18±1.16
		Methanol	11±1.16	11±0.58	10±0.58	20±7.03	17±3.61	19±3.06
Gentamicin*		µg/disc	15±1.6	13±0.1	17±0.7	14±0.08	NA	NA
Nystatin*		10µg/disc	NA	NA	NA	NA	22±0.05	20±0.1

NA:notactive,*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:<14 mm: Resistant.

3.2 Antioxidant activity

The antioxidant activity of extracts from the plant was determined by evaluating their capacity to scavenge DPPH free radicals, and the results are presented in Table 2. The highest scavenging radical activity was the methanol extract gave highest activity (45%) followed by the ethyl acetate (33%). In the case of the scavenging capacity against the DPPH radical, the values recorded in this study were higher than those reported by Santiago-Saenz *et al.*[20]. The values recorded for the scavenging capacity against DPPH of wild accessions are in ranges similar to the contents reported for species of culinary and medicinal use[21,22]. Recently, Yousef *et al.*[23] reported the radical scavenging potential of purslane leaves fresh and under different drying procedures (hot-air drying, microwave drying, and freeze-drying). Fresh purslane leaf extracts showed values of 53.23% for DPPH.

The results of the present study indicate the nutritional and functional potential of the wild purslane 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 *Portulaca oleracea*

Plantname	Organ	Extract	%RSA±SD(DPPH)
Portulacaolerace	Aerial parts	N-hexane	25±0.11
		Chloroform	10±0.03
		Ethyl acetate	33±0.17
		Methanol	45±0.04
Standard	SD	Propyl gallate	94±0.01

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

3.3 Total polyphenolic, flavonoids and tannins contents

The results of the total polyphenolic, flavonoids, and tannin contents of different extracts from the plant are presented in Table 3. Phenolic compounds are known as powerful chain-breaking antioxidants[24] and are very important plant constituents because of their scavenging ability, which is due to their hydroxyl group[25].

The highest total polyphenolic content showed that the methanolic extract (114.17±0.15mg GAE/g) flowed faster than the n-hexane extract (84.82±0.02 mg GAE/g). A previous study by Kamal *et al.*[26] of *P. oleracea* revealed higher values of total phenol content (174.5±8.5 to 348.5±7.9 mg GAE/g). The highest total flavonoid content was obtained from the ethyl acetate and methanolic extracts (499.08±0.01 and 442.24±0.01mg QE/g, respectively). Other extracts contained values ≤ 150.88±0.00 mg QE/g. It was reported that the concentration of flavonoids in plant extracts and the nature of extracted flavonoids depend on the polarity of solvents used in the extract preparation[27]. While the total tannin content in the high abundance methanolic extract (532.18±0.13 mg TAE/g) flowed, the ethyl acetate (79.61±0.02 mg TAE/g). However, it was absorbed by the two polar solvents (ethyl acetate and methanol). Variation in polyphenolic and flavonoids 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[28]. Pearson content correlation analysis revealed that the scavenging activity of the studied plant extracts was mainly attributed to the total phenolics (R2 = 0.997) and tannins (R2= 0.998) content rather than their total flavonoids (R_2= 0.09915). Several researchers have reported a significant correlation between the phenolic content and antioxidant activity of extracts[29,30].

Table 3. Total phenol content, total flavonoids and total tannins of *Portulaca oleracea.*

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
P. olerace (Aerial parts)	N- hexane	84.82±0.02	231.25±0.04	32.57±0.11
	chloroform	39.91±0.00	289.67±0.04	20.14±0.05
	Ethyl acetate	63.73±0.03	499.08±0.01	79.61±0.02
	Methanol	114.17±0.15	442.24±0.01	532.18±0.13

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

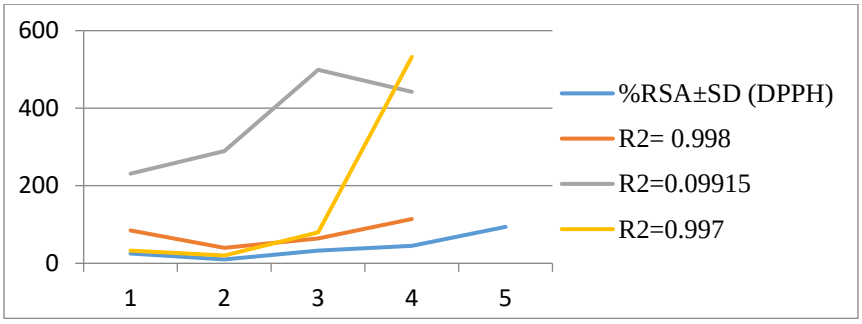

Figure 1. Correlation analysis between scavenging activity and the total polyphenolics, flavonoids, and tannin contents.
Red line = total polyphenolic content/scavenging activity.
Green line = total flavonoids content / scavenging activity. Violet line = total tannin content / scavenging activity.

4. Conclusion

Extracts of different polarities 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 activity. The majority of extracts were rich in flavonoids, while the polyphenols were mainly accumulated in the two polar extracts. Therefore, this plant could be a very beneficial source of natural bioactive agents. Further studies should be undertaken to elucidate the particular phytochemicals and their pharmacological mechanisms.

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