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Phytochemical, antibacterial screening and antioxidant activity of *Pulicaria crispa* extracts

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Abstract
Medicinal plants constitute are an effective source of both traditional and modern medicines, herbal medicine has been shown to have genuine utility. 80% of rural population depends on natural products as primary health care.
Pulicaria crispa used in folk medicine for the treatment of colds, coughs, colic, excessive sweating and as carminative.
In this study the maximum antibacterial activity was observed by The Petroleum ether Ethyl acetate, 70% methanol, ethanol respectively, while the water extract reflected no activity against all bacterial strains tested.
The chemical constituents were identified and the results exhibited the presence of Flavonoid, Tannin, Triterpenoids, Saponin, Alkaloid, Cardiac glycoside and reducing compound.
On the other hand; The ethanol, water, petroleum ether, 70% methanol extracts respectively exhibited high antioxidant activity ranging from (88±0.01, 87±0.01, 85±0.06, 84±0.02) while the ethyl acetate extract show less antioxidant activity 39±0.04.

Keywords: Phytochemical; Antibacterial; Antioxidant; *Pulicaria crispa*; *Asteraceae*.

1. Introduction
Pulicaria sp. is a genus of *Asteraceae* family. The *Asteraceae* family is one of largest families of flowering plant, with about 1,100 currently accepted genera and 25,000 species. It is characterized by the flowers being arranged in dense heads of many small florets, such as the daisy and dandelion.
The family is of worldwide distribution ,being absent only from the Antarctic mainland, and is particularly well represented in semiarid region of the tropics and subtropics, such as the Mediterranean region, Mexico, the cape province of South Africa grassland and bush land formation of Africa and montane floras throughout the world. Only in the tropical rain forests are they poorly represented.
The *Asteraceae* are of incalculably great indirect economic importance to man as major contributors to the diversity, and therefore, the stability and sustainable productivity, of the drier vegetation types throughout the world, especially in tropical and sub tropical areas. In proportion to it is size, however, the direct economic importance of the family is comparatively small. It includes food plants, sources of raw materials, medicinal and drug plants, ornamentals and succulents, and, on the debit side, weeds and poisonous plants. Many member of this chemically rich family have long been used in folk medicines [1] *Pulicaria crispa* used in folk medicine for the treatment of colds, coughs, colic, excessive sweating and as carminative [2].

2. Materials and Methods
2.1 Plant material
Pulicaria crisp (whole plant) was obtained from (Elobaied) North Kordofan, The plant was identified and authenticated by the Medicinal and Aromatic plants Research Institute (2012).

2.2 Preparation of the plant material
Plant part was cleaned, freed from dust and foreign material, and then dried under the shade and finally crushed using an electric house-hold spice grinder.

2.3 Preparation of the crude extracts
A weight of 75 grams of the coarsely powdered shade – dried sample was successively

extracted using different methods. Continuous extraction [3], Maceration and Decoction.

2.4 Phytochemical screening
Phytochemical screening were performed using standard procedures [4, 5].

2.5 Preparation of Standard bacterial suspensions
One-ml aliquots of a 24-hours broth culture of the standard organisms were aseptically distributed onto nutrient agar slopes and incubated at 37 ºC for 24 hours. The bacterial growth was harvested and washed off with sterile normal saline the suspensions were stored at 4 °C until used [6].

2.6 Testing for antibacterial Activity
The cup-plate agar diffusion method [6] was adopted to assess the antibacterial activity of the prepared extracts.
One ml of the standardized bacterial stock suspension 10^8 –10^9 C.F.U/ ml were thoroughly mixed with 100 ml of molten sterile nutrient agar which was maintained at 45 ºC. 20 ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes. The agars was left to set and in each of these plates 4 cups (10 mm in diameter) were cut using a sterile cork borer and agar discs were removed. Alternate cups were filled with 0.1 ml samples of each of the extracts using automatic microlitre pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 ºC for 18 hours.
Two replicates were carried out for each extract against each of the test organisms. Simultaneously positive controls involving the addition of petroleum ether and methanol instead of the extracts were carried out separately. After incubation the diameters of the resultant growth inhibition zones were measured, averaged and the mean values were tabulated.

2.7 Minimum Inhibitory Concentration
The minimum inhibitory concentration of the plant extracts against the sensitive organisms were determined using the agar disc method .serial dilutions of the plant extracts were prepared to obtain 20, 10, 5, 2.5, 1.25, 0,625 and 0.313 mg/ ml. Each of the inoculate (1 ml) was poured into each petri-dish and the agar was later poured and allowed to set . Wells were bored using the sterile 3 mm cork borer. Serial dilutions of the extracts were added into the marked wells. The plates were incubation at 37 ºC for 4 h. The growth was observed to determine the sensitivity of each organism using clear zones of no microbial growth. The least concentration of the plant extract that had inhibitory effect was taken as the minimum inhibitory concentration (MIC) of that plant extract against such organisms [6].

2.8 Antioxidant Activity
The DPPH radical scavenging was determined according to the method of Shimada K [7] with some modification. in 96-wells plate, the test samples were allowed to react with 2.2Di (4- tert-octylphenyl) -1- Picryl-hydrazyl stable free radical (DPPH) for half an hour at 37 °C. The concentration of DPPH was kept as I (300 µM). The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517 nm using multiplate reader spectrophotometer percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate.

3. Results
3.1 Phytochemical Screening of Plant Extracts
Phytochemical Screening of *Pulicaria crispa* whole plant revealed the presence of the following metabolites as indicated in table 1. These groups might be responsible for the observed antibacterial activity of these plant.

Table 1: The Chemical Constituents of *Pulicaria crispa* Whole plant

Type of Extract	Phytochemical	Results
Ethanol Extract	Alkaloids	+
	Flavonoids	+
	Tannins	+
	Reducing compound	+
	Cardiac glycosides	+
	Saponins	+
	Anthraquinone	_
	Triterpenoids	+

Key: (+): present, (-): absent.
The result of presence of, tannins, alkaloids, Triterpenoids in *Pulicaria crispa* was agreement with [8, 9, 10, 11].

3.2 Antibacterial Activities of Plant Extracts
The Antibacterial activities of *Pulicaria crispa* whole plant extracts were examined against four standard bacterial strains and at concentration of 10 mg/ml. Table (2), fig (1)

Table 2: Antibacterial Activities of the *Pulicaria crispa* whole plant against Standard Microorganisms

Extracts	(%) of Yield	Conc %	*E.c*	*P.s*	*S.a*	*B.s*
70% methanol	27	10%	14	11. 5	14	18
ethyle acetate	5	10%	18. 5	15. 5	17. 5	24
ethanol	9	10%	15	11	13	15
Water	26	10%	-	-	-	-
petroleum ether	3	10%	19	21	17	20.5

MIZD (mm) =Mean inhibition Zone Diameter,
Standard Microorganisms used; *Sa=Staphylococcus aureus, Bs=Bacillus subtilis. Ec=Escherichia coli, Ps=Pseudomonas aeruginosa.*

Highly Sensitive=>18 mm (MIZD). Moderate=14-18 mm (MIZD). low=<13 mm (MIZD). Resistance=<11.
The Petroleum ether extract of *Pulicaria crispa* whole plant exhibited high activities against *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and moderate activity against *Staphylococcus aureus.*
On the other hand the ethyl acetate extracts of the plant reflected a maximum activity against *B. subtilis*. But the moderate activity was observed against *S. aureus* and *Pseudomonas aeruginosa*.
The Ethanol and 70% Methanol extracts of this plant reflected a Moderate activity against all bacterial strains Tested.
The water extract of *Pulicaria crispa* exhibited no activity against all bacterial strains Tested.
The result of methanol extracts were agree with [12] in *E. coli* test and with [13] in *S. aureus* a there is no study represent the biological activity of the most extracts although it is the most common extract use traditionally. Generally Variation in results may be due to environmental factor (plant sp), method of extraction, contact time between crude material and solvent beside the quality and type of solvents used.

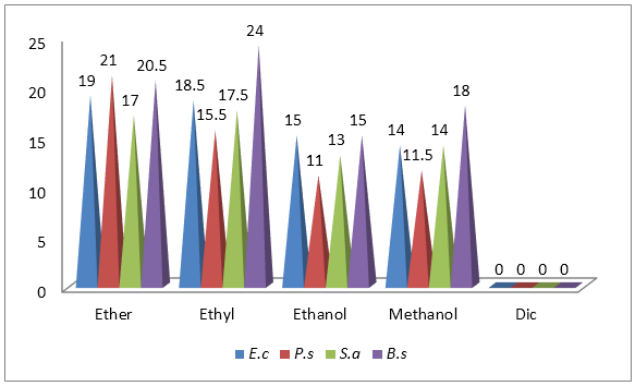

Fig. 1: Antibacterial Activity of *Pulicaria crispa* Whole plant extracts against standard bacterial strains

MIZD (mm)=Mean inhibition Zone Diameter, standard organisms used*; Sa=Staphylococcus aureus, Bs=Bacillus subtilis, Ec=Escherichia coli, Ps=Pseudomonas aeruginosa,* Dic = water extract .

3.3 Determination of the Minimum Inhibitory Concentration (MICs)

The Minimum Inhibitory Concentration of the most active extract (i.e. the Petroleum ether extract of *Pulicaria crispa*) was determined against the standard Microorganisms (*Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa*).

Table 3: The Minimum Inhibitory Concentration of the Petroleum Ether of *Pulicaria crispa*

The MIC was taken as the lowest concentration of the extract that will prevent the growth of the susceptible test bacteria.

sample	Conc %	*E.c*	*P.s*	*S.a*	*B.s*
Petroleum ether	5%	17	15	14	16
	2.50%	20	13	-	15
	1.25%	14	-	-	-
	0.63%	-	-	-	-

According to results showed in table (3) the Petroleum ether extract of *Pulicaria crispa* whole plant in concentration 5% showed the moderate antibacterial MIC against these four tested organisms. And the concentration 2.50% showed high activity against *E. coli* and moderate activity against *B. subtilis and P aeruginosa and* exhibited no activity against *S. aureus*.

The Petroleum ether extract in concentration 1.25 exhibited a moderate activity against *E. coli* and no activity against the other three bacteria.

The Petroleum ether extract in concentration 0.63% exerted no activity against all bacterial strain tested.

70% methanol extract (10 µg/ml) of *Pulicaria crispa* whole plant against *B. subtilis.* higher than (5, 10, 20,40 µg/ml) of Amoxicillin and less than (40, 20, 10 µg/ml) of Gentamicin against *B. subtilis, S. aureus*, *E. coli*.

On the other hand; Ethyl acetate extract less than (40 µg/ml) of Gentamicin against *S. a, E. coli B. subtilis* and higher than (20 µg/ml) of gentamicin against *E. coli.* and the same extract higher than (40, 20, 10, µg/ml) of Amoxicillin against *E. coli. B. subtilis.*

However the Ethanol extract of whole plant less than (40, 20, 10, µg/ml) of gentamicin and similar to (20 µg/ml) of Amoxicillin against *B. subtilis.*

Petroleum ether extract of *Pulicaria crispa* whole plant) higher than (40, 20, 10, µg/ml) of Amoxicillin against all bacterial strain tested, and less than (40, 20 µg/ml) of gentamicin against *S. a, E. coli B. subtilis*.

Table 4: Antibacterial activity of some drugs against some standard bacterial strains

Drug	Concentration (µg /ml)	MIZD (mm)		
		S. aureus	***E. coli***	***B subtilis.***
Amoxicillin	40	18	14	17
	20	15	12	15
	10	10	-	13
	5	-	-	-
Gentamicin	40	27	26	28
	20	22	20	23
	10	19	17	19
	5	10	13	14

MIZD (mm) =Mean inhibition Zone Diameter

3.4 Antioxidant activity

The antioxidant activity of different extracts of *Pulicaria crispa* whole plant have been evaluated using important parameters (%RSA±SD) free radical scavenging activity DPPH .

Table 5: DPPH radical scavenging assay of *Pulicaria crispa*

No.	Sample Cod	%RSA ± SD (DPPH)
1	PE	85±0.06
2	EtOH	88±0.01
3	Ethyl	39±0.04
4	water	87±0.01
5	MeoH	84±0.02

As indicated in table (4), the most potent activity on the DPPH scavenging activity was observed by ethanol extract (88±0.01) while the ethyl acetate extract exhibited a less activity as compared with other extracts.

4. Discussion

This activity may be related to the secondary metabolites which detected as coumarins, tannins and alkaloids *on P. crispa* [8]. Investigation of the flavonoids constituents of *Pulicaria crispa*, result in the isolation and identification of four flavonoids, quercein, quercetin-3-o-glucoside, quercetin-7-o glycoside and quercetin-3-methyl ether [14].

The presence of these metabolites explains the various uses of this plant in traditional medicine.

Alkaloids, comprising a large group of nitrogenous compounds, are widely used as cancer chemotherapeutic agents [15, 16] alkaloids also interfere with cell division The presence of alkaloids in *Pulicaria crispa* may be responsible for antibacterial activity as recorded in this study.

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