

## Phytochemical constituents, antioxidant activity and antimicrobial potential of *Pulicaria incisa* (lam.) DC as a folk medicinal plant

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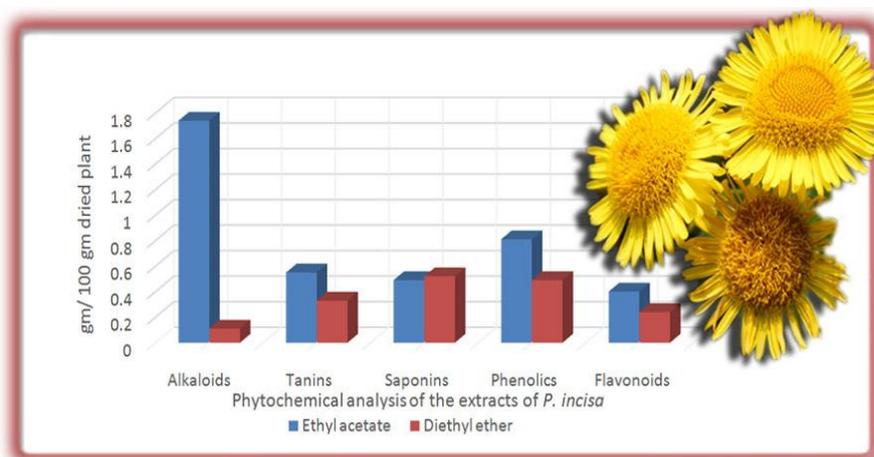
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### GRAPHICAL ABSTRACT



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### ABSTRACT

The species belonging to the genus *Pulicaria* are well known for their traditional uses in folklore medicine, active chemical constituents and pharmacological activities. Therefore, this study aimed to determine the active constituents, antioxidant activity and antimicrobial potential of *Pulicaria incisa* (lam.) DC extracts. Ethyl acetate and diethyl ether extracts of the dried aerial parts of *P. incisa* were prepared. The active secondary constituents (alkaloids, phenolics, flavonoids and tannins) were quantitatively determined. The active constituents in the ethyl acetate extract were higher than those of diethyl ether extract. The ethyl acetate extract expressed higher antioxidant activity in terms of diphenyl picryl hydrazyl (DPPH<sup>•</sup>) radical scavenging assay in comparison with ascorbic acid as a reference standard. The antimicrobial activity analysis revealed that the ethyl acetate extract expressed antimicrobial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Erwinia carotovora* and *Candida albicans* while that of diethyl ether expressed activity against *Bacillus subtilis*, *Erwinia carotovora* and *Candida albicans*. In conclusion the antioxidant and anti-microbial activity of the studied extracts of *P. incisa* may be attributed to its richness with the medicinally active metabolites.

## Introduction

Genus *Pulicaria* of the family Asteraceae has around one hundred species distributed in Europe, North Africa and Asia [1]. *Pulicaria incisa* (lam.) DC is a medicinal plant known as wild tea or Shay Gebeli and is used by natives of upper Egypt and by Bedouins as a decoction, tea substitute and for treatment of heart diseases [2]. It is characterized by its aromatic smell and essential oil content. *P. incisa* was reported to be used in Sudanese traditional medicine as a tonic, antispasmodic, hypoglycemic in addition to its usage as an ingredient of a local perfume. The traditional medicinal properties of this plant have been approved through scientific research as several studies were done on it revealed its hypo-cholesteremic, antimicrobial, anticancer and antioxidant properties [2-10].

It is common in Egypt throughout the Sahara, including the Red Sea region and Sinai. It is grown in non-saline wadi beds and has a wide ecological range from sandy to gravelly soils but sand-loamy soils are preferable [11].

The evolution of antibiotic-resistant microbes like *Staphylococcus aureus* decrease the number of antibiotics used for the treatment of clinical infections and this has motivated the search for novel antimicrobial drugs from natural sources [12].

This study aims to evaluate the phytochemical constituents, antioxidant and antimicrobial potential of two different extracts prepared from *P. incisa*.

## Materials and methods

### Plant material

The aerial parts of *P. incisa* were collected from their natural habitat in Saint Catherine protectorate during the period from August to mid-September 2018. The samples botanical identities were identified and authenticated according to Bolous [13]. Two kilos of

aerial parts of *P. incisa* were air dried in shade for 20 days and grinded to be ready for use.

### Preparation of the plant extracts

Extraction of the target species was carried out using two different extraction solvents including ethyl acetate and diethyl ether. 10 grams of the dried plant was extracted by shaking for three hours at 250 rpm with 200 ml of ethyl acetate and the same was done for diethyl ether. Subsequently, the extracts were filtered then evaporated to dryness using rotary evaporator.

### Determination of the active secondary metabolites

#### Total phenolics

Phenolics content was measured using Folin Ciocalteu assay described by Lin and Tang [14] and determined as gram gallic acid equivalent (GAE)/ 100-gram air dried.

#### Total flavonoids

Flavonoids content was measured using aluminum chloride assay described by Chang *et al.* [15] and determined as gram quercetin equivalent (QE)/ 100-gram air-dried plant.

#### Total alkaloids

Alkaloids content was measured using 1,10-phenanthroline method described by Singh *et al.* [16] and determined as gram alkaloids / 100-gram air-dried plant.

#### Total tannins

Tannin content was determined by Vanillin hydrochloride method of Sadasivam, & Maickam [17] and determined as gram gallic acid equivalent/ 100-gram air-dried plant).

## Evaluation of antioxidant activity

### DPPH• Assay

The efficacy of the studied extracts against DPPH• free radical was determined according to the assay described by Kitts *et al* [18]. The antioxidant activity was calculated as the amount of antioxidant needed to diminish the initial DPPH• concentration by 50% (IC<sub>50</sub>). Ascorbic acid was used as a standard reference compound.

### Screening of the antimicrobial activity

#### Disc diffusion assay

The antimicrobial potential of *P. incisa* extracts was determined using filter paper disc assay [19].

#### Tested organisms

*Bacillus subtilis* DMS-1088, *Klebsiella pneumoniae* ATCC-10031, *Staphylococcus aureus* ATCC-6538, *Staphylococcus epidermidis* EMCC number-1353<sup>‡</sup>, *Escherichia coli* ATCC-10536, *Erwinia carotovora* ATCC-15713<sup>™</sup> and one pathogenic fungal strain, *Candida albicans* EMCC number-105.

## Results and Discussions

Previous phytochemical studies on *Pulicaria* species reported the ability of them to synthesize phenolics, flavonoids, tannins and terpenoids. Such compounds from *Pulicaria* found to have potential bioactivities and to be a promising source for new drugs and higher value products [20]. These could be helpful for the protection of humans against chronic diseases. For instance, phenolics have many biological features such as antibacterial, antiinflammation, antiaging, anticarcinogen and cardiovascular protection. Flavonoids are potent water-soluble free radical scavengers useful in preventing oxidative cell damage and possess anticancer activity in addition to their antimicrobial activity. Alkaloids protect against several chronic diseases [21-24].

The phytochemical screening of ethyl acetate and diethyl ether extracts of *P. incisa* revealed the presence of medicinally active metabolites like alkaloids, tannins, phenolics, flavonoids, glycosides and terpenoids. These active compounds were quantitatively analyzed and the results obtained revealed that ethyl acetate extract was higher in its active constituents than diethyl ether extract and the ethyl acetate extract was rich in its active metabolites in considerable amounts than those of diethyl ether extract (Table 1).

**Table 1-** The estimated secondary metabolites in *P. incisa*

Extract	Ethyl acetate	Diethyl ether
TA	1.74	0.11
TT	0.55	0.33
TS	0.49	0.52
TP	0.81	0.49
TF	0.40	0.24

TA= total alkaloids expressed as gram alkaloids / 100-gram air-dried plant, TS= total saponins expressed as gram saponins / 100-gram air-dried plant, TT= total tannins expressed as gram of gallic acid equivalents/100-gram air-dried plant, TP = Total phenolics expressed as gram of gallic acid equivalents/100-gram air-dried plant, TF = Total flavonoids expressed as gram catechin equivalent per 100-gram air-dried plant.

The phenolics and flavonoids present in the prepared extracts in variable quantities are well known as potent antioxidant compounds [24-26] and could be contributed to the antioxidant activity of the studied extracts. The antioxidant activity of *P. incisa* extracts are reported in Table 2.

**Table 2-** Antioxidant activity of the extracts prepared from *P. incisa*

Extract	Ethyl acetate	Diethyl ether	Ascorbic acid
IC <sub>50</sub>	0.009	0.14	0.023

The antioxidant activity was measured using DPPH• radical assay. The antioxidant scavenging activity for DPPH• is attributed to its hydrogen-donating ability [27]. Ascorbic acid was used as the standard reference compound (Table 2). The strongest activity was detected in the ethyl acetate that was higher in its antioxidant activity than the reference standard and diethyl ether extract. The antioxidant activity attributed to the presence of the active phytochemical constituents as phenolics, flavonoids, Alkaloids, saponins and tannins as reported in Table 1.

### Antimicrobial Assessment

There were several studies which have been done for screening the antimicrobial potential of *pulicaria* species where *P. jaubertii* and *P. stephanocarpa* showed antimicrobial potential against *Bacillus subtilis*, *P. inuloides* against *Streptococcus pneumoniae* while essential oils from *P. incisa* exhibited activity against *Streptococcus pneumoniae*, *E. coli*, *Bacillus subtilis*, *Aspergillus fumigatus*, *Syncephalastrum racemosum* and *Geotricum candidum*[28, 29]. It has been reported in the

literature that the main constituents express the antimicrobial activity of the plant extracts are phenolics, flavonoids, terpenoids, essential oils, alkaloids, lectins, and polypeptides[30].

The antimicrobial activity of *P. incisa* ethyl acetate and diethyl ether extracts were tested using *Bacillus subtilis* DMS-1088, *Klebsiella pneumoniae* ATCC-10031, *Staphylococcus aureus* ATCC-6538, *Staphylococcus epidermidis* EMCC number-1353<sup>t</sup>, *Escherichia coli* ATCC-10536, *Erwinia carotovora* ATCC-15713<sup>tm</sup> and one pathogenic fungal strain, *Candida albicans* EMCC number-105. The tested ethyl acetate and diethyl ether extracts expressed significant inhibition zones in a dose-dependent manner. The antimicrobial activity analysis revealed that the ethyl acetate extract expressed antimicrobial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Erwinia carotovora* and *Candida albicans* while that of diethyl ether expressed activity against *Bacillus subtilis*, *Erwinia carotovora* and *Candida albicans* and all the obtained results were compared with the antibiotic Streptomycin as illustrated in Table 3.

**Table 3-** Antimicrobial activity of *P. incisa* ethyl acetate and diethyl ether extracts

Microorganisms	Diameter of inhibition zone (mm)		
	Ethyl acetate	Diethyl ether	Streptomycin
<i>Erwinia carotovora</i>	9.5	9	17
<i>Klebsiella Pneumoniae</i>	-	-	-
<i>Escherichia coli</i>	-	-	-
<i>Bacillus subtilis</i>	-	7.5	14
<i>Staphylococcus aureus</i>	11	-	10
<i>Staphylococcus epidermidis</i>	8.5	-	18
<i>Candida albicans</i>	13	10	12

Zone of inhibition measured in mm and included filter paper disk diameter (6mm); “-”: no inhibition.

## Conclusion

The obtained results illustrated that *P. incisa* could be regarded as a potential source of useful drugs. The phytochemicals in this plant justifies its traditional medicinal uses. The results of this study in addition to those from previous studies could be considered as a reference to the antioxidant and antimicrobial activity of *P. incisa* with biologically active and stable components. Thus, a scientific foundation to use this plant in medicine can be profound to improve the local user's healthcare.

## Competing interests

We declare that we have no competing interests.

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