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RESEARCH ARTICLE

Screening of Phytochemicals and *In vitro* Antioxidant activity of *Evolvulus alsinoides* L.

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Abstract

Present study attempts to evaluate the qualitative analysis, antibacterial and antioxidant efficacy of the petroleum ether, dichloromethane, ethyl acetate, ethanol and aqueous extracts of *Eolvulus alsinoides* L. Antibacterial activity of the crude extracts of *E. alsinoides* was tested against gram-positive *Staphylococcus aureus* and gram-negative *Salmonella typhi, Acinetobacter baumannii, Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa* using agar well diffusion method. Aqueous extract showed maximum inhibitory activity against *A. baumannii* (13 and 16 mm) followed by *E. coli, S. aureus* and *K. pneumoniae*. Phytochemical analysis indicated the presence of flavonoids, alkaloids, triterpenes, glycosides, terpenoids, anthraquinones, phytosterol, polyphenol, tannins and sterols. Radical scavenging activities of petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts of *E. alsinoides* ranged from 5.11-28.87%, 8.91-.29.85%, 14.85-45.70%, 17.98-53.38% and 35.89-75.16% respectively. Among all the extracts, aqueous showed higher activity followed by ethanol, ethyl acetate, chloroform and hexane. The findings of the study indicated a reasonable antibacterial potential and significant total antioxidant activity, thus supporting its traditional medicinal practices.

Keywords: Qualitative analysis, plant extracts, antibacterial, antioxidant efficacy, radical scavenging activity.

Introduction

In India around 20,000 medicinal plant species have been recorded but more than 500 traditional communities use about 800 plant species for curing different ailments of human beings (Kamboj, 2000). Currently 80% of the world population depends on plant-derived medicine as the first line of primary health care for human beings since it has no side effects (Chopra *et al.,* 1956). Hence, alternative medicines are available for those who do not want conventional medicine or who cannot be helped by conventional medicine. Historically pharmacological screening of compounds of natural or synthetic origin has been the source of innumerable therapeutic agents. Random screening as a tool in discovering new biologically active molecules has been most productive in the area of antibiotics (Gerhartz *et al.,* 1985). *Evolvulus alsinoides* L. is a perennial herb belonging to the family *Convolvulaceae* with a small woody and branched root stock (Austin, 2008). This plant is used in traditional medicine in East Asia, India, Africa and Philippines to cure fever, cough, cold, venereal diseases, azoospermia, adenitis and dementia, nootropic and anti-inflammatory activity (Singh, 2008). Goyal and Singh (2005) reported its use in the treatment of neurodegenerative diseases, asthma and amnesia. Pre-clinical research has justified its ancient claim as brain tonic (Singh, 2008). Several other uses reported for this plant include its ability to boost memory and improve intellect, immunomodulatory, adaptogenic as well as antioxidant properties (Sethiya *et al.,* 2009).

Against these backdrops, the present study was attempted to evaluate the qualitative analysis, antibacterial and antioxidant efficacy of the petroleum ether, dichloromethane, ethyl acetate, ethanol and aqueous extracts of *Evolvulus alsinoides* L.

Materials and methods

Plant collection: Evolvulus alsinoides was collected in a sterile polythene bag from marunduvalmalai hilly areas, Pothaiyadi village, Agastheeswaram taluk at Kanyakumari District, Tamil Nadu, India. Morphological characters of the selected plant were recorded at the time of plant collection in the field notebook by using hand lens.

Preparation of plant extract: Evolvulus alsinoides were collected from the field and washed thoroughly with running tap water and rinsed in sterile distilled water by following the method of Akowuah and Ismail (2005). The washed plant materials were shade dried at room temperature for 10 d. The shade dried plant materials were then ground well into fine powder. The powder thus obtained was used for the extraction of bioactive compounds.

Solvent extraction: The plant powder was extracted with different solvents, such as petroleum ether, chloroform, ethyl acetate and ethanol in a Soxhlet apparatus for 8 h.

The extracts obtained from the Soxhlet apparatus were concentrated to dryness under reduced pressure and the residue was dissolved at the concentration of 1 mg/mL in DMSO (Dimethyl Sulfoxide) (Poonkothai *et al.,* 2005) and stored in air tight container at 4°C.

Aqueous extraction: A total of 25 g finely powdered plant material was infused in distilled water until completely saturated. The extract was then filtered using muslin cloth and the filtrate was concentrated. The filtrate was collected in a sterile flask and refrigerated (Harborne, 1998). The extract was concentrated using a rotary evaporator (Heidolph laborata, Germany) at various temperatures under reduced pressure.

Qualitative analysis of bioactive compounds: Ethanol and aqueous extract of *E. alsinoides* was subjected to preliminary screening of bioactive compounds namely alkaloids, flavonoids (Harborne, 1973), triterpenes, glycosides (Oloyed, 2005), terpenoids (Harborne, 1998), anthraquinones, phytosterol, polyphenol, tannins (Trease and Evans, 1989) and sterols (Harborne, 1991).

Antibacterial activity: Bacterial isolates were cultured on nutrient agar and incubated at 37°C for 24 h and the microorganisms were repeatedly sub-cultured in order to obtain pure isolation. Morphological and biochemical reactions were carried to ascertain proper identification. They were inoculated into nutrient agar slants and stored at 4°C. Overnight broth culture of the respective bacterial strains was adjusted to turbidity equivalent to 0.5 McFarland standards. (0.2 mL culture of organisms was dispensed into 20 mL sterile nutrient broth and incubated for 24 h and standardized at 10^5-10^7 CFU/mL adjusting the optical density to 0.1 at 600 nm.

Antibacterial activity of aqueous extract of *E. alsinoides* was determined using agar well diffusion assay. Human clinical pathogenic bacterial strains namely *Salmonella typhi* (MTTC 734), *Acinetobacter baumannii* (MTCC 9819), *Staphylococcus aureus* (MTTC 7443), *Klebsiella pneumoniae* (MTCC 109), *Escherichia coli* (MTCC 443) and *Pseudomonas aeruginosa* (MTTC 4727) were obtained from Gen Bank, Institute of Microbial Technology Sector, Chandigarh-160036, India. Different solvent extracts were used for antibacterial assay by well diffusion against human pathogens using Muller Hinton Agar (MHA) in sterile petridishes (Sanaa *et al.,* 2003). When the media solidified, 0.1 mL of active growth culture was poured over feeder layer and spread evenly by sterile spreader. A 6 mm diameter well was made by sterile cork borer and each well received different concentrations (50, 100 and 150 µL/mL) of crude extract. They were dissolved in 4% DMSO (Dimethyl sulfoxide). Appropriate control was maintained and incubated at 37°C for 48 h. After incubation, the inhibition zone was measured (mm).

Antioxidant assay of aqueous extract E. alsinoides:
DPPH free radical scavenging activity: DPPH free radical scavenging activity of each sample was determined using the UV/vis spectrophotometer according to the method described by Leong and Shui (2002). Briefly, a 0.1 mM solution of DPPH in methanol was prepared. The initial absorbance of DPPH in methanol was measured at 515 nm and did not change throughout the period of assay. An aliquot (40 µL) of an extract (with appropriate dilution, if necessary) was added to 3 mL of methanol DPPH solution. The free radical-scavenging activity (FRSA) was calculated using the formula: FRSA = $[(A_c–A_s)/A_c] \times 100$, Where, A_c is the absorbance of the control and A_s is the absorbance of the tested sample after 60 min.

Determination of chelating effects of ferrous ions: The chelation of ferrous ions by the extracts was estimated by method of Dinis *et al.* (1994). Briefly, 50 µL of 2 mM $FeCl_2$ was added to 1 mL of different concentrations of the extracts (0.2, 0.4, 0.8, 1.6 and 3.2 mg/mL). The reaction was initiated by the addition of 0.2 mL of 5 mM ferrozine solution. The mixture was vigorously shaken and left to stand at room temperature for 10 min. The absorbance of the solution was thereafter measured at 562 nm. The percentage inhibition of ferrozine Fe^{2+} complex formation was calculated as $[(A_0- A_s)/A_s] \times 100$, where A_0 was the absorbance of the control and A_s was the absorbance of the extract/standard. Na_2EDTA was used as positive control.

Results

The phytochemical analysis of the ethanolic and aqueous extracts indicated the presence of major phyto-compounds, including phenolics, alkaloids, glycosides, flavonoids and tannins (Table 1).

Table 1. Phytochemical screening of *E. alsinoides* extracts.

Constituents	Ethanolic extract	Aqueous extract
Alkaloids	-	+
Anthraquinones	-	-
Flavonoids	+	+
Glycosides	-	+
Phytosterol	-	-
Polyphenol	+	-
Sterols	+	-
Tannins	+	+
Terpenoids	-	-
Triterpenes	+	-

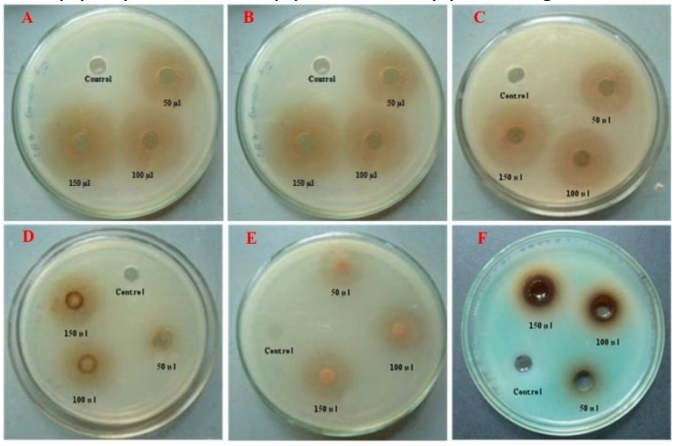
Antibacterial activity: In the present study, the aqueous extract of *E. alsinoides* displayed antibacterial activity against *S. typhi*, *A. baumannii*, *S. aureus, K. pneumoniae*, *E. coli* and *P. aeruginosa* (Fig. 1). Results were compared with different concentrations of 50, 100 and 150 µg/mL of the extracts. Each test was performed in triplicates.

A further objective was to gain knowledge about aqueous extract (150 µg/mL) to appraise a possible application of this material as antibacterial agent. The comparative study of different concentrations and its antibacterial activities are shown in Table 2.

Table 2. Antibacterial activity of aqueous extract of *E. alsinoides* against human pathogens.

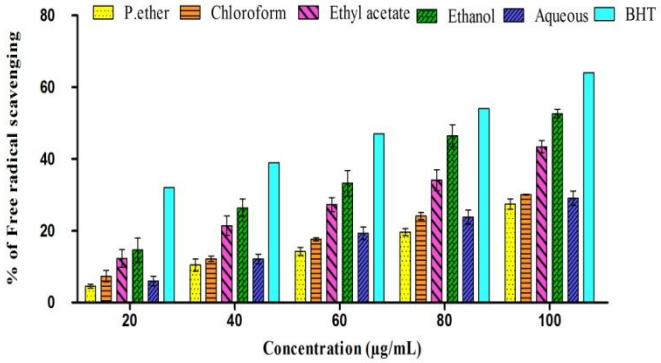
Microorganisms	Zone of inhibition (mm)		
	50 µg/mL	100 µg/mL	150 µg/mL
S. typhi	8	11	14
A. baumannii	10	13	16
S. aureus	10	11	12
K. pneumoniae	7	9	11
E. coli	8	10	13
P. aeruginosa	6	8	9

Fig. 1. Antibacterial activity of aqueous extract of *E. alsinoides* against (A) *S. typhi*, (B) *A. baumannii*, (C) *S. aureus,* (D) *K. pneumoniae*, (E) *E. coli* and (F) *P. aeruginosa.*



DPPH radical scavenging activity: Scavenging effects of hexane, chloroform, dichloromethane, ethyl acetate extracts from *E. alsinoides* on DPPH radicals was found to be increased with the increase in concentration at 20-100 µg/mL. Scavenging activities of the petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts of *E. alsinoides* on DPPH radical ranged from 5.11-28.87%, 8.91-29.95%, 14.85-45.70%, 17.98-53.38% and 35.89-75.16% respectively. The results indicated that petroleum ether, chloroform, dichloromethane and ethyl acetate extracts, showed good, moderate and poor activities at various concentrations tested. Among all the extracts, aqueous extract showed higher activity followed by ethanol, ethyl acetate, chloroform and petroleum ether (Table 3 and Fig. 2).

Fig. 2. DPPH radical scavenging activity of *E. alsinoides* extracts.



Metal chelating activity: In this assay, the chelating agents disrupt the ferrozine-Fe^{2+} complex, thus decreasing the red color. The antioxidant compounds interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and capture ferrous ions before the formation of ferrozine. In the present study, the chelating activity of the hexane, chloroform, dichloromethane and ethyl acetate extracts from *E. alsinoides* at five different concentrations (0.063, 0.125, 0.250, 0.500, and 1.000 mg/mL) toward ferrous ions were investigated. The chelating abilities of various extracts of *E. alsinoides* increased as the concentration increased. The strongest chelating effect (76.23%) was obtained with ethyl acetate extract at 1.0 mg/mL. At this concentration, the lowest chelating effect (7.19%) was exhibited by the petroleum ether extract. All of the extracts at the concentration of 1000, 500, 250, 125 and 0625 µg/mL showed significant activity (Table 4 and Fig. 3).

Fig. 3. Metal chelating activity of *E. alsinoides* extracts.

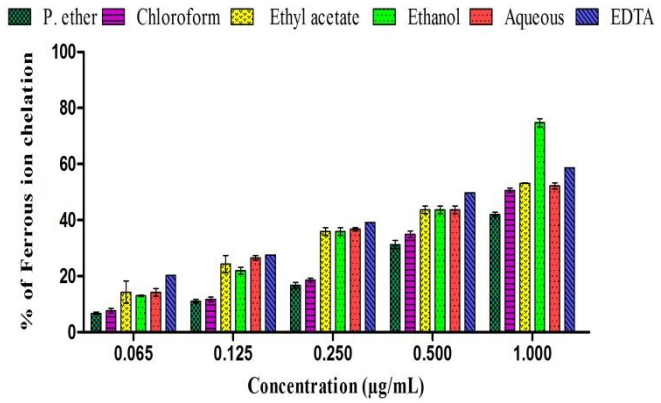


Table 3. DPPH radical scavenging activity of *E. alsinoides* extracts.

Conc. (µg/mL)	(%) Radical scavenging assay					Standard BHT (%)
	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Aqueous	
100	28.87	29.95	45.70	53.38	75.16	64.00
80	18.64	23.18	36.96	49.50	61.05	54.00
60	13.20	18.15	29.20	36.71	56.43	47.00
40	8.74	12.95	24.00	25.82	47.93	39.00
20	5.11	8.91	14.85	17.98	35.89	32.00

Table 4. Metal chelating activity of *E. alsinoides* extracts.

Conc. (µg/mL)	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Aqueous	EDTA
1000	42.77	51.34	69.19	76.23	53.31	72.73
500	32.73	36.11	49.76	58.19	44.92	49.76
250	17.73	19.31	36.51	46.38	37.29	39.24
125	11.68	12.52	23.23	29.39	27.30	27.50
0.625	7.19	8.53	12.71	18.27	15.57	20.23

Discussion

In the present study, aqueous extract of *E. alsinoides* displayed antibacterial activity against *S. typhi*, *A. baumannii*, *S. aureus, K. pneumoniae*, *E. coli* and *P. aeruginosa.* Dash *et al*. (2002) reported antibacterial activity of ethanolic extract of *E. alsinoides* against *P. aeruginosa* (23 mm), *E. coli* (26 mm) *S. aureus* (but inactive) at 40 mg/mL in his study. Omogbai and Eze (2011) reported antibacterial activity of the aqueous and ethanolic extracts of *E. alsinoides* against *K. pnuemoniae* (24 and 38 mm), *P. aeruginosa* (6 and 33 mm), *S. typhi* (0 and 30 mm), and *E. coli* (12 and 26 mm) at 1025 mg/mL. The role of free radicals and tissue damage in diseases, such as atherosclerosis, heart failure, neurodegenerative disorders, aging, cancer, diabetes mellitus, hypertension and several other diseases, is gaining a lot of recognition (Shimizu *et al.,* 2001). Both reactive oxygen species (ROS), as well as reactive nitrogen species (RNS), are well recognized as playing a dual role as both are deleterious and beneficial species, in that they can be either harmful or beneficial to living systems. Antioxidant activities exhibited by methanolic extracts may be due to the presence of phenolic substances and flavonoids or diterpenes. A number of studies have investigated the ability of flavonoid-rich fraction to act as antioxidants (Aviram, 2004; Sudheesh and Vijayalakshmi 2004). Flavonoids can directly react with superoxide anions and lipid peroxyl radical (Torel *et al.,* 1986) and consequently inhibit/break the chain of lipid peroxidation. This radical scavenging activity of extracts could be related to the antioxidant nature of polyphenols/flavonoids, thus contributing to their electron/hydrogen donating ability. Chelating of Fe^{2+} by extracts was estimated by the method of Dinis *et al.* (1994). The transition metal ion, Fe^{2+} possess the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions, even starting with relatively non-reactive radicals (Aboul-Enein *et al.,* 2003). *Evolvulus alsinoides*, the most active extract interfered with the formation of ferrous and ferrozine complex, suggesting that it has chelating activity and captures ferrous ion before ferrozine.

Conclusion

The phytochemical analysis of the crude extracts of *E. alsinoides* indicated the presence of major phyto-compounds, including phenolics, alkaloids, glycosides, flavonoids and tannins.

Aqueous extract of *E. alsinoides* displayed antibacterial activity against *S. typhi*, *A. baumannii*, *S. aureus, K. pneumoniae*, *E. coli* and *P. aeruginosa.* Scavenging effects of hexane, chloroform, dichloromethane, ethyl acetate extracts from *E. alsinoides* on DPPH radicals was found to be increased with the increase in concentration at 20-100 µg/mL. *Evolvulus alsinoides*, the most active extract interfered with the formation of ferrous and ferrozine complex, suggesting that it has chelating activity and captures ferrous ion before ferrozine. Further studies are needed on the isolation and characterization of individual compounds of this edible plant to elucidate their various antioxidant mechanisms.

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