

Anti Bacterial and Anti Oxidant Activities of *Evolvulus alsinoides* Linn

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Abstract: Plants are very important sources of potential useful raw materials as natural chemotherapeutic agents. Shankhpushpi is botanically termed as *Evolvulus alsinoides*; the extracts have exhibited antioxidant, anti-ulcer, and immunomodulatory activities. The present work is to investigate the invitro therapeutic potential activities of methanolic extract of whole plant of *Evolvulus alsinoides* on the antimicrobial activity against five clinical pathogenic bacterial strains viz., *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumonia* and *Salmonella typhi*, using agar well diffusion assay and Anti oxidant activity using 1-1-Diphenyl-2-picryl-hydrazyl (DPPH) radical. Extract inhibited IC₅₀: 40.2. Total phenolic content: 28.4 mg/ml & Total flavonoid content: 20.2 µg /ml. *Evolvulus alsinoides* (MIC): 512.5mg/ml possess 30 to 40 % inhibition, the antibacterial activity of the herbal extracts was more pronounced on the gram- negative bacteria *Klebsiella pneumonia* than the gram-positive bacteria *Staphylococcus aureus*. The free radical scavenging activity of methanolic activity of whole plant of *Evolvulus alsinoides* increase in a concentration dependent manner, and posses statistically significance DPPH free radical scavenging & Anti microbial activities

Keywords: *Evolvulus alsinoides*, total phenolic and flavonoid content, DPPH free radical Scavenging & Anti microbial activities

I. Introduction

Medicinal herbs are a rich source of antimicrobial agents. A wide range of medicinal plant parts are used for extract as raw drugs and possess varied medicinal properties.^{1, 2} Primitive people learned by trial and error to distinguish useful plants with beneficial effects from those that were toxic or non-active and also which combinations or processing methods had to be used to gain consistent and optimal results.³ In spite of the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care.⁴ In comparison with modern medicine, herbal medicines cost less, are more often used to treat chronic diseases and the occurrence of undesirable side effects seems to be less frequent. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with side effects and have great therapeutic potential to heal many infectious diseases.⁵ Taking into consideration the large potential of plants as sources for antimicrobial drugs, many systematic investigations were taken into consideration and screened in different parts of the world.^{6,7}

The importance of the reactive oxygen species (ROS) has attracted increasing attention over the last decade. Reactive oxygen species include free radicals such as super oxide anion radicals, hydroxyl radicals, non free radicals such as hydrogen peroxide and singlet oxygen, along with various forms of activated oxygen. They are involved in various physicochemical processes and disease such as aging, cancer and activity atherosclerosis.⁸

The preliminary phytochemical screening carried out in *E. alsinoides* contains some secondary metabolites such as glycosides, alkaloids, poly phenols, carbohydrates, amino acids and proteins, saponins, volatile oil, flavonoids and tannins.⁹ The plant contains alkaloids such as betaine, shankhapushpine, B-sitosterol and evolvine. Fresh plant contains volatile oil.

These compounds help brain stimulation and increase the ability to concentrate. Early phytochemical studies of this species resulted in the isolation and identification of chemical constituents such as triacontane, pentatriacontane.

Four unidentified alkaloids A, B, C and evolvine have also been described. The present study planned on Methanolic extract of whole plant of *Evolvulus alsinoides* for antioxidant potential and Anti microbial activities, which possess prominent immunomodulatory response and for treating various infectious diseases

II. Materials And Methods

(1) Plant materials and extraction

The plant material was collected from the local rural area of Tumkur district in Karnataka state, India. The plant materials were identified and authenticated. The Plant material were air-dried and pulverized in course powder and was loaded in the Soxhlet apparatus for defating with petroleum ether and followed by extraction with methanol. The methanolic extract of *Evolvulus alsinoides* was subjected to fractionation and dry concentrated extract were used for the study

(2) Analytical test for TLC

Alkaloid analyses were done by using the Solvent system: Ethyl acetate: Formic Acid: Acetic Acid: Water – Ratio 50:5.5:5.5: 13.5, Yellow-Brown colored spots were detected in day-light and UV (256 nm) Flavonoid analyses were done by using the Solvent system: Chloroform: Methanol- Ratio 42.5: 7.5, Yellow colored spots in Day-light and UV.

(3) Total Phenolic compounds

The method (Slinkard K., et al, 1977; Ragazzi and Veronese, 1973) downscaled to 1 mL final volume was followed to determine the amounts of total phenolics in the test samples. The test samples (each 100 / L) were mixed with 500 /L of 1:10 Folin-Ciocalteau's reagent followed by the addition of Na₂CO₃ (400 / L, 7.5%).^{10,11} After incubating the reaction mixture at 24 °C for 2 h, the absorbance at 765 nm was recorded. Gallic acid monohydrate was used as the standard. The total phenolic contents as gallic acid equivalents (μg of GAE)/mg dry weight of the samples

(4) Total flavanoid content

The determination of the total flavanoid content (TFC) was carried out, briefly, 2.5 ml of each extract solution was mixed with 2.5 ml AlCl₃ reagent in ethanol 90% and allowed to stand for 40 min at room temperature. After that, the absorbance of the mixture at 415 nm was measured with a SHIMADZU Multispect-1501 spectrophotometer.¹²

Ethanol 90% (2.5 ml) plus sample solution (2.5 ml) was used as a blank. Rutin was used as a reference compound. The TFC for each extract [as μg rutin equivalents (RE) / mg of extract] was determined on the basis of the linear calibration curve of rutin (absorbance versus rutin concentration)

(5) DPPH free radicals scavenging activity

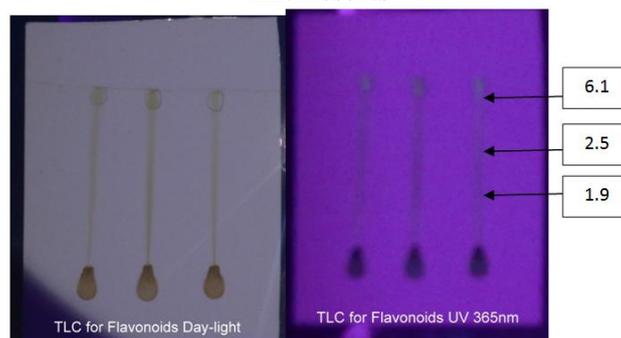
The free radical scavenging activity of the different fractions of *Evolvulus alsinoides* was measured using DPPH, 1 ml of each fraction of *Evolvulus alsinoides* and the reference compound in various concentration (50,100, 250 & 500 $\mu\text{g}/\text{ml}$) was added to 1 ml of 0.1mM solution of DPPH in methanol. After 30 min., absorbance was measured at 517 nm using spectrophotometer. 0.01 mM solution of DPPH in methanol was used as control, whereas Quercetin was used as reference material. All tests were performed in triplicate. Percentage inhibition was calculated by using following formula.¹³

$$\% \text{ Inhibition} = \frac{\text{control absorbance} - \text{test absorbance}}{\text{Control absorbance}}$$

(6) Anti bacterial Activity:

Antibacterial activity of methanolic extract of *Evolvulus alsinoides* against different test organism was determined using a modified Kirby-Bauer disc diffusion method.¹⁴ Briefly, 100 μl of the test bacteria were grown in 10 ml of fresh media until they reached a count of approximately 10⁸ cells/ml. 100 μl of the microbial suspension was spread onto nutrient agar plates.

III. Results



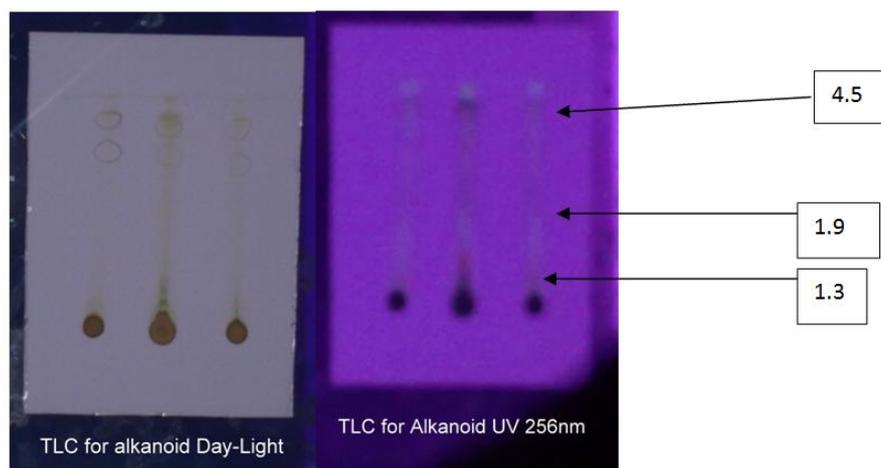


Fig: 1- TLC analysis

Table: 1. Anti oxidant potential of Methanolic extracts of *E. alsinoides*

Plant material	TPC (mg/g)	TFC (µg/mg)	DPPH (IC ₅₀)
<i>Evolvulus alsinoides</i>	28.4 ± 0.128	20.2 ± 0.212	40.2 ± 0.21

Note: TPC: Total Phenolic compounds. TFC: Total flavanoid content
DPPH: free radicals scavenging activity (Anti oxidant activity).

Table: 2. The Minimum Inhibitory Concentrations of Methanolic extracts of *E. alsinoides* on Bacterial isolates

SERIAL NO	ISOLATES	MTCC. No	MIC
1	<i>Bacillus cereus</i>	1305	512.5
2	<i>Staphylococcus aureus</i>	3160	512.5
3	<i>Klebshiella pneumonia</i>	39	32
4	<i>Escherichia coli</i>	443	64
5	<i>Salmonella typhii</i>	1254	16

Table: 3. The Antibacterial Activities of the Methanolic extracts of *E. Alsinoides* on Bacterial strains

Conc. mg/ml	Zone of Inhibition (mm) of Bacterial Isolates				
	B. cereus	S. aureus	K. pneumonia	E. coli	S. typhi
512.5	08	05	34	23	24
256	00	00	30	18	21
128	00	00	26	12	16
64	00	00	24	08	10

The result of antibacterial activity of methanolic extract of *Evolvulus alsinoides* against different test organism have been tabulated in table - 2 and 3.

At a concentration of 512.5mg/ml, the highest zone of clearance was obtained from methanol extract against *Klebshiella pnuemoniae* with a diameter of 34mm. This was followed by *Salmonella typhi* (24mm) and *Escherichia coli* (23mm) respectively. The lowest zone of inhibition at this concentration was 5mm against *Staphylococcus aureus*.

IV. Discussions

The TLC analysis conducted in this experiment shows the presence of few alkaloids and flavonoids in the methanolic extract of *Evolvulus alsinoides* (table -1). This plant is used in Asia, Africa, and Philippines to cure fever, cough, cold, venereal disease, azoospermia, adenitis, and dementia, nootropic and anti inflammatory activity reported its use in the treatment neurodegenerative disease asthma and amnesia.^{15, 16} Pre- clinical researches has justified its ancient claim's brain tonic.¹⁵ Several other uses reported for this plant include its ability to boost memory and improve intellect, immunomodulatory, adaptogenetic as well as antioxidant properties.¹⁷ Plant phenolics constitute one of the major groups of compounds responsible for antioxidant behavior, as well as for antimicrobial effects.

Flavonoids, this diverse and widespread group of natural compounds are the most important natural phenolics. They possess a broad spectrum of biological activities, including radical scavenging properties and antibacterial effect (table -1). The study plant *E. alsinoides* contains alkaloids: betaine, shankhpushp and evolvine. Fresh plant contains volatile oil. It also contains a yellow neutral fat, an organic acid and saline substances.

Scopoletin, scopolin, umbelliferone, 2-methyl-1,2,3,4-Hydrophobic molecules can pass through cell wall of gram-positive bacteria easier than the gram-negative bacteria because cell wall of the gram-positive bacteria contained only peptidoglycan (table-2,3).^{18,19} These results agree with those reported that the antimicrobial effects of essential oil constituents are dependent on their hydrophobicity.²⁰

V. Conclusion

The results revealed that the methanolic extract of *Evolvulus alsinoides* are potent antimicrobials against all the microorganisms studied, since the plant extracts were rich in Alkaloid and flavonoids, it indeed possess potent antioxidant activities

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