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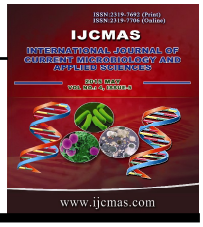

Original Research Article

Investigation of Antibacterial Activities of *Evolvulus alsinoides* (L.) against Clinical Pathogens

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Keywords

Antimicrobial activity, *Evolvulus alsinoides*, Pathogenic bacteria and medicinal plants

A B S T R A C T

Plants are very important sources of potential useful raw materials for the new development of natural chemotherapeutic agents. Hence, the present investigation was carried out the antimicrobial activity of various parts of plant of *Evolvulus alsinoides.* The antimicrobial activity of *E. alsinoides* was evaluated against four clinical pathogenic bacterial strains viz., *Staphylococcus aureus, Bacillus cereus, Escherichia coli* and *Pseudomonas aeruginosa,* using agar well diffusion assay. The results revealed that the extracts are potent antimicrobials against for all the strains studied. The results indicated that the plants extracts showed antibacterial activities at variable degrees against clinical bacteria, with inhibition zone varying diameter from 10 to17 mm. In root extract maximum inhibition zone diameter was obtained in *S. aureus and* in *B. cereus* with diameter 17 mm 14 mm, respectively. For all the tested microorganisms, root extract showed maximum antibacterial activity. Hence, among the various parts, the root part can be used for treat bacterial diseases. The antibacterial activity of the herbal extracts was more pronounced on the gram-positive bacteria *S. aureus* than the gram-negative bacteria *E. coli*. The data obtained in antimicrobial activity of this plant indicates the promising potential in the treatment of various diseases as a medicine and also justified the ethnic uses of *Evolvulus alsinoides* against various infectious diseases.

Introduction

An antimicrobial is a compound that kills or inhibits the growth of microbes such as bacteria. Such a compound is said to have antibacterial activity (Jagessar *et al.*, 2008). 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 (Gisesa, 2004; Egwaikhide and Gimba, 2007). 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 (Jagessar *et al.*, 2008). In spite of the great advances observed in modern medicine in recent decades, plants still make

an important contribution to health care (Ravikumar *et al.,* 2010). 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 (Jagessar *et al.,* 2007). 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 (Nascimento *et al.,* 2000; Ahmed and Beg, 2001).

One such plant which claims various medicinal properties is *Evolvulus alsinoides* one of the popular and important medicine plant in India. All parts of *Evolvulus alsinoides* possess valuable medicinal properties. This plant is widely used in ayurveda. In ayurvedic literature *Evolvulus alsinoides* is known as vishnukrantha, which has been told to have 'Medhya' 'Smritivardhaka' and 'Buddhivardhaka' (Memory enhancer) action and is categorized in the 'Samjnasthapana dravyas'. It grows in open and grassy places throughout almost all of India and subtropical countries of the world. The whole herb is used medicinally in the form of decoction or infusion in doses of 2-4 ounces with cumin and milk and used in fever, nervous debility and less of memory also in syphilis, scrofula. In fevers attended with diarrhea or indigestion a decoction of the drug with the *Ocimum sanctum* is administered (Nadkarni, 2009). Therefore, extracts of these herbs possibly have *antimicrobial* activity. Hence, the present study has been carried out to evaluate the antimicrobial activity of the extracts of various parts (root, stem, leaves and Flowers) of E. *alsinoides* against the four human pathogens and to find out the particular microorganisms for which the herbal extracts are active.

Materials and Methods

Description of plant

E. alsinoides L. (dwarf morning glory) belonging to the family Convolvulaceae is a perennial herb with a small woody and branched rootstock. Its branches are annual, numerous, more than 30 cm long, often prostrate, slender and wiry with long hairs. Leaves are small, entire, elliptic to oblong, obtuse, apiculate, base acute and densely hairy. Flowers mostly solitary in upper axils, Corolla blue rotate and broad funnel shaped.

Sample collection and preparation

The sample leaves, stem, root and flowers of *Evolvulus alsinoides* were used in the study. The sample of the authenticated herbal plants were then collected and air-dried for twelve weeks to obtain constant weight. The dried sample was cut into smaller pieces and ground into fine particles with a grinder. The powdered sample was bagged in black plastic bags and stored in an air-tight container for further work.

Extraction

About 50g of plant part (i.e. root, stem, leaf and flowers) were taken and they were extracted with 50ml of methanol in sterilized air tight amber coloured container**.** The extraction was carried out in a ¼ liter flask. Extractions were done and the extracts were concentrated to about one-sixth of the original volume at 60°C under reduced pressure using a rotary evaporator. The extracts were air-dried for three weeks to a constant weight and kept in air-tight containers for further work.

Test bacterial strains

The Pathogenic bacteria were isolated from clinical samples and collected from Bose Clinical Lab, Madurai. They were identified by different biochemical tests. All the bacterial strains used were maintained as pure cultures in slants and stored in refrigerated conditions. The list is given below:

1. *Bacillus cereus* (ATCC10870)
2. *Staphylococcus aureus* (ATCC33862)
3. *Escherichia coli* (ATCC13762) and
4. *Pseudomonas aeruginosa* (ATCC27853)

Antibacterial assay test

The antibacterial activity of plant samples were determined by agar well diffusion methods (Kirby-Bauer) is a relatively swift and easily prompted the antibacterial activity. The samples were tested against four pathogenic organisms. The suspension of bacteria was grown in nutrient broth medium.

Test organisms were dispersed over the surface of agar plates. A small amount of sample is gently pushed over the nutrient agar plate inoculated with bacterial cells for intimate contact of the sample. The plates were incubated at 37°C for 18–24 h.

Data collected

The antibacterial activity of the herbs against four pathogens was obtained by measuring the diameters of the inhibition zones and compared them with that of the control drug Amikacin. Antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by the herb extracts. Results obtained in this study were expressed as mean inhibition zone (mm) ± S.D of three replicates.

Result and Discussion

The antimicrobial potential of the experimental plant was evaluated according to their zone of inhibition against various pathogens and the results (zone of inhibition) were presented in table 1, fig. 1 and Plate 1–4. The results indicated that the plants extracts showed antibacterial activities at variable degrees against clinical bacteria, with inhibition zone varying diameter from 10 to17 mm. In root extract maximum inhibition zone diameter was obtained in *S. aureus and* in *B. cereus* with diameter 17 mm 14 mm, respectively. The results revealed that the extracts are potent antimicrobials against all the microorganisms studied. For all the tested microorganisms root extract showed maximum antibacterial activity in *S. aureus.*

According to World Health Organization (2004) more than 80% of the world's population relies on the traditional medicines for their most important health requirements. The medicinal value of the plants lies in few chemical substances responsible for several definite physiological actions in human. Such chemical group includes alkaloid, flavinoids, tannins and phenolic compounds (Singh, 2012). Awareness of the chemical constituents of plants is pleasing, not only for the invention of curative agents but also for the renaissance of the lost information about these valuable plants (Mojab *et al.,* 2003).

E. alsinoides L. is used mainly in traditional medicine of East Asia. The plant is used in Ayurveda as a brain tonic in the treatment of neurodegenerative diseases, asthma and amnesia. In Sri Lanka, roots and stem extract of the plant are used to treat dysentery and depression. Leaves are recommended for asthma and mental disturbances. Decoction of roots, thrice a

day, is consumed in Eastern Ghats of Andhra Pradesh, India for three days for curing cough and cold (Rajaqkaruna *et al.,* 2002). According to an ethno botanical survey conducted among Kani/ Kanikaran ethnic groups in Southern Western Ghats of India, whole plant of *E. alsinoides* is used for the treatment of venereal diseases (Ayyanar and Ignacimuthu, 2005). In Uttara Kannada district of Karnataka, *E. alsinoides* is used as spermopiotic (Hegde *et al.,* 2006). The Valaiyan community of Piranmalai hills, Tamilnadu consumes leaf juice *of E. alsinoides* internally for three days for fever (Sandhya *et al.,* 2006).

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 (Singh, 2008). Goyal and Singh (2005) reported its use in the treatment neurodegenerative disease asthma and amnesia. Pre- clinical reaserch has justified its ancient claimas brain tonic (Singh, 2008). Several other uses reported for this plant include its ability to boost memory and improve intellect, immunomodulatory, adaptogenetic as well as antioxidant properties (Sethiya *et al.,* 2009).

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. The study plant *E. alsinoides* contains alkaloids: betaine, shankhapushpine 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-butanetetrol, ferulic acid esters with alcohols C14-C17 and palmitic, stearic, oleic, 8-methyldecanoic and heptadecanoic acids have been reported (Cervenka *et al.,* 2006). 2,3,4-trihydroxy-3- methylbutyl 3-[3-hydroxy-4-(2,3,4-trihydroxy-2-methyl butoxy)-phenyl]-2-propenoate (1) and 1,3-di-*O*-caffeoyl quinic acid methyl ester, caffeic acid, 6-methoxy-7-*O*-β-glucopyranoside coumarin, 2-C-methyl erythritol, kaempferol-7-O-β-glucopyranoside, kaempferol-3-O-β-glucopyranoside and quecetine-3-O-β-glucopyranoside were reported from *n*-BuOH soluble fraction from the ethanol extract of *E. alsinoides* (Gupta *et al.,* 2007). The phytochemical composition of the plant may be the reason for higher the antibacterial activity against different bacterial strains tested.

Results of this study demonstrated that the gram-negative bacteria (*Bacillus cereus & Staphylococcus aureus)* were more resistant to all the plant extract than gram-positive bacteria (*E. coli & P. aeruginosa), b*ecause lipopolysaccharide (LPS) layer of gram-negative bacteria in outer membrane have a high hydrophobicity which acts as a strong permeability barrier against hydrophobic molecules (Smith-Palmer *et al.,* 1998). 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 (Nikaido and Vaare, 1985; Lambert *et al.,* 2001). These results agree with those reported by Lan-ciotti *et al.* (2004) who confirmed that the antimicrobial effects of essential oil constituents are dependent on their hydrophobicity.

The results revealed that the extracts are potent antimicrobials against for all the microorganisms studied. For all the tested microorganisms root extract showed

maximum antibacterial activity. Hence, the root part can be used for treat bacterial diseases. The results of this work suggest that the plant have a broad spectrum of antimicrobial activity and which can be used as an alternative for antibiotics. Moreover, this plant extract should be investigated *in vivo* to better understand their safety, efficacy and properties.

Table.1 Antibacterial activity of methanolic extract of different parts of *Evolvulus alsinoides* against test organisms

Sl.No	Name of the Pathogen	Activity (mm)			
		Root	Stem	Leaf	Flower
1.	*S. aureus*	17	12	10	12
2.	*B. cereus*	14	13	14	13
3.	*E.coli*	13	R	R	R
4.	*P. aeruginosa*	10	R	10	R

R : Resistant

Fig.1. Antibacterial activity of differet parts of *Evolvulus alsinoides* against test organisms

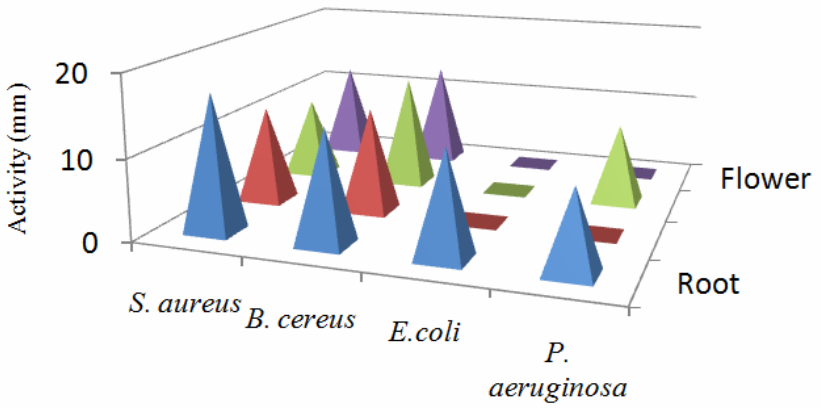
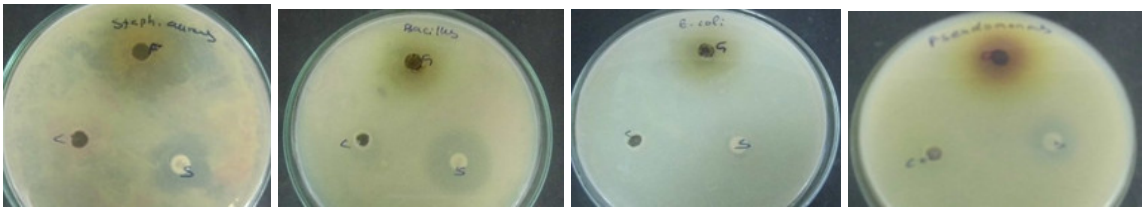
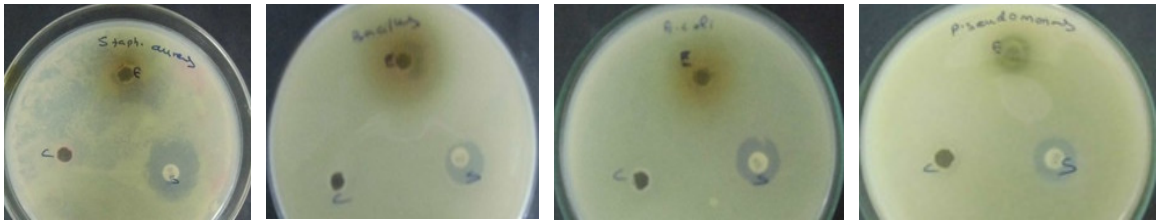


Plate.1 Antibacterial activities of root extract of ***Evolvulus alsinoides***



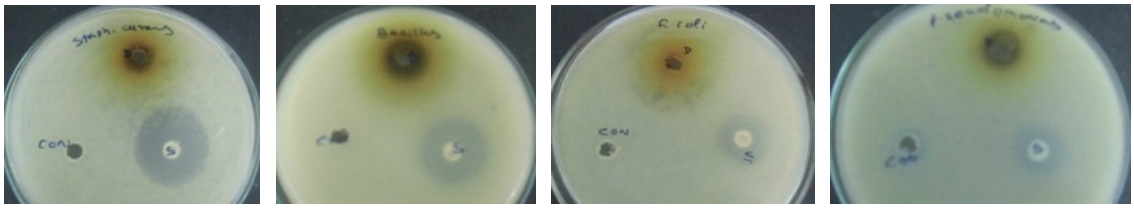
(a) (b) (c) (d)

Plate.2 Antibacterial activities of stem extract of ***Evolvulus alsinoides***



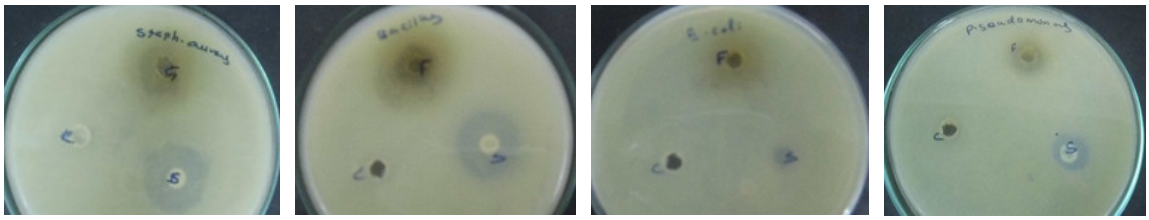
(a) (b) (c) (d)

Plate.3 Antibacterial activities of leaf extract of ***Evolvulus alsinoides***



(a) (b) (c) (d)

Plate.4 Antibacterial activities of flower extract of ***Evolvulus alsinoides***



(a) (b) (c) (d)

a : *S. aureus* b:*B.cereus* c: *E. coli* d: *P. aeruginosa*

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