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ASSESSMENT OF PHYTOCHEMICAL CONSTITUENTS, TRACE METALS AND ANTIMICROBIAL EFFICACY OF HOLY PLANT *EVOLVULUS ALSINOIDES*, SOUTHERN INDIA

R. Anbarasu*, G. Selvan, S. Baskar and V. Raja

Department of Physics, Thanthai Hans Roever College, affiliated to Bharathidasan University, Perambalur – 621 212, Tamil Nadu, India.

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***Correspondence for Author**
R. Anbarasu
Department Of Physics, Thanthai Hans Roever College, Affiliated to Bharathidasan University, Perambalur – 621 212, Tamil Nadu, India.

ABSTRACT

Medicinal plants and their extracts deserve special attention because of the important influence they have to human health. Even though there are easily contaminated with surrounding environmental factors, which have certain bioactive compounds that are act as drug against many diseases. The aim of this study was to achieve the qualitative and quantitative determination of phytochemicals and metallic elements in medicinal plant such as *Evolvulus alsinoides* using standard respective methods. The phytochemical screening of the crude extract revealed the presence of steroids, triterpenes, alkaloids, phenols, flavonoids and tannins. Metal content in the powdered medicinal plants was in descending order: Zn > Fe > Cu > Cd > Cr = Ni = Pb. The average mean concentrations of Cd, Cr, Cu, Fe, Ni, Pb and Zn were 0.08, BDL, 0.24, 0.29, BDL, BDL and 0.46 mg kg^{-1}, respectively. The methanol extract of *E.alsinoides* were analyzed for in-vitro antimicrobial activity against certain pathogens and the zone of inhibition were compared with positive (such as Methicillin – 10 mcg and Itraconazole – 10 mcg) and negative controls (DMSO). In antimicrobial activity, the methanol extract samples against bacterial strains were most effective against *Enterococcus fecalis* (B2) while the smaller effect was noticed from *Micrococcus luteus* (B4). In fungi, the test sample was effective against *Trichophyton rubrum* (F4).

KEYWORDS: *Evolvulus alsinoides*, Antimicrobial activity, Phytochemistry, Trace metals, Methanol extract.

INTRODUCTION

Natural products once served humankind as the source of all drugs, and higher plants provided most of these therapeutic agents. Today, natural products (and their derivatives and analogs) still represent over 50% of all drugs in clinical use, with higher plant-derived natural products representing ca. 25% of the total (Koperuncholan et al. 2010). The World Health Organization estimates that 80% of the people in developing countries of the world rely on traditional medicine for their primary health care, and about 85% of traditional medicine involves the use of plant extracts. This means that about 3.5 to 4 billion people in the world rely on plants as sources of drugs Phytochemicals are compounds that occur naturally in plants. They contribute to the color, flavour and smell of plants. In addition, they form part of a plant's natural defence mechanism against diseases.

In other hand, the role of cement pollutants causing injury to plants either by direct toxic effect or modifying the host physiology rendering it more susceptible to infection (Gupta and Mishra, 1994). In severe case of pollution, the injury symptoms were expressed as foliar necrosis or completely disappearance of the plant (Ramesh et al. 2014). have also previously studied the impact of air pollution on plants with reference to foliar anatomical and biochemical changes by experimenting on various sensitive plants. Increased concentrations of the above pollutants cause progressive reduction in the photosynthetic ability of leaves, closure of leaf stomata and, mainly, a reduction in growth and productivity of plants (Larcher 1995). These dust particulates are causing large scale deforestation destruction of biota and other natural resources.

Evolvulus alsinoides (slender dwarf morning-glory) is flowering plant from the family *Convolvulaceae*. It has a natural pantropical distribution encompassing tropical and warm-temperate regions of Australasia, Indomalaya, Polynesia, Sub-Saharan Africa and the Americas. The species inhabits a wide range of habitats, from marshland and wet forests to deserts. A number of varieties and subspecies are recognised. It may become a weed in some situations. It is one of the plants included in Dasapushpam, the ten sacred flowers of Kerala. This herb used in traditional medicine of East Asia for its purported psychotropic and nootropic properties (Amritpal Singh 2008). Although such claims are not medically verified. Chemical compounds isolated from *E. alsinoides* include scopoletin, umbelliferone, scopolin and 2 – methyl - 1, 2, 3, 4-butanetetrol (Cervenka 2008). In present study focus the

phytochemical constituent, trace metal concentration and antimicrobial activity of methanolic extracts from the aerial parts of *E.alsinoides*.

MATERIALS AND METHODS

Plant Material

The plant materials were collected from Tiruchirappalli district of Tamil Nadu in India during the period of April to May 2015. The shade dried *E. alsinoides* powders (100 g) were successively extracted with ethanol by soxhlet apparatus and is used as test sample for further studies.

Phytochemical screening

Qualitative analysis

The solvent extracts were subjected to routine qualitative chemical analysis to identify the nature of phytochemical constituents present in sample. Steroids: A 3 ml of test solution and minimum quantity of chloroform was added with 3-4 drops of acetic anhydride and one drop of concentrated H_2SO_4. Purple color thus formed changes into blue or green color indicating the presence of steroids. Triterpenoids: A 3 ml of test solution was added with a piece of tin and 2 drops of thionyl chloride. Formation of violet or purple colour indicates the presence of triterpenoids. Reducing Sugars: A 3 ml of test solution was added with a 2 ml of Fehling's reagent and 2 ml of water. Formation of reddish orange color indicates the presence of reducing sugar. Sugars: A 3 ml of the test solution was added with very small quantity of anthrone reagent and a few drops of concentrated H_2SO_4 and heated. Formation of green or purple color indicates the presence of sugars. Alkaloids: A 3 ml of test solution was taken with 2N HCl. Aqueous layer formed was decanted and then added with one or a few drops of Mayer's reagent. Formation of white precipitate or turbidity indicates the presence of alkaloids.

Phenols: A 3 ml of test solution in alcohol was added with one drop of neutral ferric chloride (5%) solution. Formation of intense blue color indicates the presence of phenols. Flavonoids: A 3 ml of test solution in alcohol was added with a bit of magnesium and one (or) two drops of concentrated HCl and heated. Formation of red or orange color indicates the presence of flavonoids. Saponins: A 3 ml of test solution was added with water and shacked. Formation of foamy lather indicates the presence of Saponins. Tannins: A 3 ml of test solution was added with water and lead acetate. Formation of white precipitate indicates the presence of tannins. Anthroquinones: A 3 ml of test solution was added with magnesium acetate.

Formation of pink color indicates the presence of anthroquinones. Amino Acids: A 3 ml of test solution was added with 1% ninhydrin in alcohol. Formation of blue or violet color indicates the presence of amino acids. Catechins: A 3 ml of test solution in alcohol was added with Ehrlich reagent and a few drops of concentrated HCl. Formation of pink color indicate the presence of catechins.

Quantitative analysis of phytoconstituents

The chlorophyll pigments in the leaves were estimated following the method of Arnon. After pre-cleaning, weighted fresh leaf material was homogenized and extracted thrice in chilled 80% acetone (v/v). The volume of the acetone extract was made up to a known one and the optical density was read at 645 nm and 663 nm wavelengths on a spectrophotometer. The concentration of the chlorophyll pigments was calculated and is expressed in mg/g fresh weight. Amino acids were estimated by ninhydrin method which is calorimetrically measured at 570nm. Proteins were estimated by bradford method and the absorbance was measured at 595nm against blank/ sample. Carbohydrates were estimated by anthrone method which can be measured by using colorimetrically at 620 nm (or) by using a red filter. All the trials were performed thrice and the mean values were presented.

Trace metal analysis

The plant leaves of *E.alsinoides were* carefully removed and washed with sterile distilled water. The cleaned leaves were dried in shadow area and were grinned with agate mortar and pestle. The powered plant samples were stored in sterile plastic container. The 1 g of powdered plant sample was treated with aqua-regia mixture in Teflon bomb and was incubated at 140 °C for 2-3 days. After incubation, the reaction mixture was filtered with whatman No.1 filter paper. Then, the extraction was tested for trace metals (Fe, Cu, Zn, Pd, Cd, Cr and Ni) analysis by the 797 VA Computrace voltametry, Metrohm. To avoid the contamination, the devices were rinsed with acidified water (10% HNO_3) and weighted to dissolve metals before analysis. And, all the equipments and containers were soaked in 10% HNO_3 for 24 h then rinsed thoroughly in de-ionized water before use. Also find the below detectable limit of the instruments.

Testing of antimicrobial activity

The test strains were: *Aeromonas liquefaciens* MTCC 2645 (B1), *Enterococcus faecalis* MTCC 439 (B2), *Micrococcus luteus* NCIM 2871 (B3), *Salmonella typhimurium* NCIM 2501 (B4), *Candida albicans* MTCC 1637 (F1) and *Cryptococcus* sp. MTCC 7076 (F2). The

cultures were obtained from MTCC, Chandigarh and NCIM, Pune, India. Microbial strains were tested for antimicrobial sensitivity using the disk diffusion method (Vignesh et al., 2012a; Vignesh et al., 2012b; Lakshmi praba et al., 2013). The antibacterial and antifungal activities of test samples were analyzed against certain microorganisms on muller hinton agar (MHA) and potato dextrose agar (PDA), respectively (Vignesh et al., 2013; Vignesh et al., 2015a). The solvent extracted samples were dissolved in concentrated DMSO. A sterile cotton swab was used to inoculate the bacterial suspension on surface of agar plate. The three different concentrations (2.5, 5 & 10 mg/ml) of sample were poured into disk and placed on agar plates, separately. For negative control study, the DMSO was used. The plates were incubated at 37±1°C for 24–48 h (for bacteria) and 25 ±1°C for 48-72 h (for fungi) (Vignesh et al., 2015b). After incubation, the zone of inhibition was measured with ruler. The assays were performed in triplicate and the average values are presented. Methicillin – 10mcg (for bacteria) and Itraconazole – 10mcg (for fungus) was used as positive control (Anitha et al., 2011; Pandiyarajan et al., 2013). All the media, standard discs and sterile disc were purchased from Hi-Media (Mumbai, India).

RESULTS AND DISCUSSION

Phytochemical constituents of Secondary metabolites

Plant secondary metabolism produces products that aid in the growth and development of plants but are not required for the plant to survive. Secondary metabolism facilitates the primary metabolism in plants. This primary metabolism consists of chemical reactions that allow the plant to live. In order for the plants to stay healthy, secondary metabolism plays a pinnacle role in keeping all the of plants' systems working properly. A common role of secondary metabolites in plants is defence mechanisms. They are used to fight off herbivores, pests, and pathogens. Although researchers know that this trait is common in many plants it is still difficult to determine the precise role each secondary metabolite. Secondary metabolites are used in anti-feeding activity, toxicity or acting as precursors to physical defence systems. The present study revealed that the medicinal plant such as *E.alsinoides* contains bioactive compounds. The phytochemical constituents were screened by qualitative and quantitatively methods and the results are presented in Table 1 and 2. In steroids analysis, purple color thus formed changes into blue or green color indicating the presence of steroids. Similarly, based on the presence or absence of colour change indicate positive and negative results. The polar solvent methanol used to screen *E.alsinoides* were contain steroids, triterpenes, alkaloids, phenols, flavonoids and tannins. Commonly, secondary metabolites play an important role

in plant defence against herbivory. A steroid is a type of organic compound that contains a characteristic arrangement of four cycloalkane rings that are joined to each other. Examples of steroids include the dietary lipidcholesterol, bile acids, the sex hormonesestradiol and testosterone and the anti-inflammatory drug dexamethasone. Steroids are a class of organic compounds with a chemical structure that contains the core of gonane or a skeleton derived therefrom. Usually, methyl groups are present at the carbons C-10 and C-13 – an alkyl side-chain at carbon C-17 may also be present. Hundreds of distinct steroids are found in plants, animals and fungi. All steroids are made in cells either from the sterolslanosterol (animals and fungi, see below right) or from cycloartenol (plants). Both lanosterol and cycloartenol are derived from the cyclization of thetriterpene squalene (Lanosterol biosynthesis).

Terpenes are released by trees more actively in warmer weather, acting as a natural form of cloud seeding. The clouds reflect sunlight, allowing the forest to regulate its temperature. (Adam, David 2008) The aroma and flavour of hops, highly desirable in some beers, comes from terpenes. Of the terpenes in hops Mycenae, β-pinene, β-caryophyllene, and α-humulene are found in the largest quantities. (Glenn Tinseth 2010) Terpenes are used by termites of the Nasutitermitinae family to attack enemy insects, through the use of a specialized mechanism called a fontanellar gun (Nutting et al. 1974). Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, and animals, and are part of the group of natural products (also called secondary metabolites). Many alkaloids can be purified from crude extracts by acid-base extraction. Many alkaloids are toxic to other organisms. They often have pharmacological effects and are used as medications, as recreational drugs, or in entheogenic rituals. Examples are the local anaesthetic and stimulant cocaine, the psychedelic psilocin, the stimulant caffeine, nicotine, the analgesic morphine, the antibacterial berberine, the anticancer compound vincristine, the antihypertension agentreserpine, the cholinomimeric galantamine, the spasmolysis agent atropine, the vasodilator vincamine, the anti-arrhythmia compound quinidine, the anti-asthma therapeutic ephedrine, and the antimalarial drug quinine. Although alkaloids act on a diversity of metabolic systems in humans and other animals, they almost uniformly invoke a bitter taste. (Rhoades, David 1979).

Maximilian Nierenstein studied natural phenols and tannins (Drabble et al. 1907) found in different plant species. Working with Arthur George Perkin, he preparedellagic acid from

algarobilla and certain other fruits in Perkin and Nierenstein 1905. He suggested its formation from galloyl-glycine by Penicillium in (Nierenstein 1915). Tannase is an enzyme that Nierenstein used to produce m-digallic acid from gallotannins. (Nierenstein 1932) He proved the presence of catching in cocoa beans in (Adam et al. 1931). He showed in (Nierenstein 1945) that luteic acid, a molecule present in the myrobalanitannin, tannin found in the fruit of Terminalia chebula, is an intermediary compound in the synthesis of ellagic acid.

Steroids along with phospholipids function as components of cell membranes. Steroids such as cholesterol decrease membrane fluidity. Terpenes are released by trees more actively in warmer weather, acting as a natural form of cloud seeding. The clouds reflect sunlight, allowing the forest to regulate its temperature. Alkaloids are antibacterial berberine, the anticancer compound vincristine, the anti-hypertensionagen treserpine, the cholinomimeric galantamine, the spasmolysis agent atropine, the vasodilator vincamine, the anti-arrhythmia compound quinidine, the anti-asthma therapeutic ephedrine, and the antimalarial drug quinine. Although alkaloids act on a diversity of metabolic systems in humans and other animals, they almost uniformly invoke a bitter taste (Koperuncholan and Ahmed John. 2011). Flavonoids are one class of secondary plant metabolites that are also known as Vitamin P or citrin (Koperuncholan et al. 2010). These metabolites are mostly used in plants to produce yellow and other pigments which play a big role in colouring the plants.

Some of the trace metals are essential for plant growth whereas many of them affect the plant physiology. Especially, the role of trace metal pollutants causing injury to plants either by direct toxic effect or modifying the host physiology rendering it more susceptible to infection.[20] which leads to affects the photosynthesis process, growth and their efficiency.[21] The mean concentrations of metals such as Cd, Cr, Cu, Fe, Ni, Pb and Zn in plant sample were BDL, 0.02, 0.32, 0.89, BDL, BDL and 0.54 mg kg^{-1}, respectively (Table 3). The antimicrobial activity of *E.alsinoides* was examined with various microorganisms using the well diffusion test. The results of the antimicrobial activities are summarized in Table 4. The three tested concentrations such as 2.5, 5 & 10 mg/ml produce zone of inhibition on MHA and PDA plates for bacteria and fungi, respectively. In the present study, higher (10 mg/ml) concentration of sample got greater sensitivity than (2.5 & 5 mg/ml) lower concentration in most of the microorganisms. In bacteria, the Methanol extract samples were most effective against *Enterococcus fecalis* (B2) while the smaller effect was noticed from *Micrococcus luteus* (B4). In fungi, the test sample was effective against *Trichophyton rubrum* (F4). There

is no antimicrobial activity in solution devoid of sample used as a vehicle control (concentrated DMSO), reflecting that antimicrobial activity was directly related to the sample.

Table 1: Qualitative phytochemical constituent of *E.alsinoides*

Phytochemical Constituents	Methanol
Steroids	+
Triterpenes	-
Reducing sugars	-
Sugars	-
Alkaloids	-
Phenolics	-
Catechins	-
Flavonoids	-
Saponins	-
Tannins	+
Anthraquinones	-
Amino acids	-

+ = Present; - =Absent

Table 2: Quantitative phytochemical constituent of *E.alsinoides.*

Biochemical constituents	*E.alsinoides* (mg/g)
Chlorophyll A	0.219
Chlorophyll B	0.852
Total Chlorophyll	1.071
Amino acid	155.0
Protein	2.120
Carbohydrate	1.509
Phenol	0.028

Table 3: Concentration of trace metals in *Evolvulus alsinoides.*

Sampling Site	Sample Name	Cd	Cr	Cu	Fe	Ni	Pb	Zn
Tiruchirappalli Tamil Nadu	*Evolvulus alsinoides*	0.08	BDL	0.28	0.74	BDL	BDL	0.46

BDL – Below detectable limit

Table 4: Antimicrobial activity of the different solvent extracts of *Evolvulus alsinoides* leaves.

S. No	Test Microorganisms	Methanol extract mg/ml			PC	Diseases	Route of Transmission
Bacteria		**2.5**	**5.0**	**10.0**	**10 mcg**		
1.	*Aeromonas liquefaciens* B1	8	11	12	14	Wound Infections / Gastroenteritis	Water / Food
2.	*Enterococcus fecalis* B2	12	13	16	8	Endocarditis / Epididymal Infections	Water / Food
3.	*Klebsiella pneumoniae* B3	11	12	14	28	Acute diarrhoea / Dysentery	Water / Food
4.	*Micrococcus luteus* B4	9	12	16	38	Skin & Pulmonary infections	Soil / Water / Air / Food
5.	*Salmonella typhimurium* B5	10	13	15	0	Typhoid	Water / Food
6.	*Vibrio cholarae* B6	11	14	16	16	Cholera	Water / Food
Fungi							
7.	*Candida albicans* F1	10	12	17	10	Skin infection / Gastrointestinal tract Infection	Air / Wound / Soil / Water
8.	*Cryptococcus* sp. F2	12	14	16	9	Bronchiectasis / Endophthalmitis.	Air / Wound / Soil / Water
9.	*Microsporum canis* F3	12	14	15	9	Tinea capitis /Ringworm	Air / Wound / Soil / Water
10.	*Trichophyton rubrum* F4	11	15	18	7	Tinea corporis / Tinea pedis	Air / Wound / Soil / Water

PC - Positive Control (Using antibiotic disc; Bacteria – Methicillin (10mcg/disc); Fungi – Itraconazole (10mcg/disc) Samples – 2.5, 5, 10 mg/ml (well)

CONCLUSION

This study concluded that the presence of steroids, triterpenes, alkaloids, phenols, flavonoids, and tannins are helps to the antimicrobial activity and is effective than positive control except B1, B3 and B4. The presence of heavy metals indicated that the plant was contaminated as well as which is resistant to the trace metal. Interestingly, the secondary metabolites of *E. alsinoides* were not affected. In this endeavour, traditional herbal medicines must perforce be granted the benefits of modern science and technology to serves further global needs. The *E.alsinoides* may act as an alternative antibiotic in near future.

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