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Research Article

Antimicrobial Activity and Phytochemical analysis of *Evolvulus alsinoides* against HIV Secondary Pathogens

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Abstract

Background: The antimicrobial activities of medicinal plants have long been exploited by scientific community against the diseases causing microorganisms. Routine use of antibiotic agents developed microbes as drug resistant organisms making it as a critical issue in case of treatments. So the detection of new natural compounds with antimicrobial activity is considered to be important. Although hundreds of plant species have been said to have antimicrobial properties, the vast majority have not been adequately evaluated. The present research works focus to develop new therapeutic agents or drug from selected medicinal plant of *Evolvulus alsinoides* against HIV Secondary pathogenic organisms, based on the review of literature, problems associated with treatment of pathogenic organisms and drug resistant patterns of the pathogenic organisms.

Results: The isolated bacterial cultures from secondary infection of HIV patient's samples were identified as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* based on their Morphology, Staining, Motility and Biochemical test. The isolated fungal cultures were identified as, *Aspergillus niger*, *Penicillium sp*, *Mucor sp*, and *Candida albicans*, based on their growth and LCB staining. *Evolvulus alsinoides* has shown to have several compounds that play a great function in its antimicrobial activity.

Conclusion: There is a need to develop alternative agents for the control of pathogenic bacterial and fungal diseases in HIV infected persons. There is a good reason to support that the secondary metabolites of plants have evolved to protect them from attack by microbial pathogens. Hence, products from plants have great potential as sources of novel bioactive compounds for controlling pathogenic microorganisms. The treatment of various infectious diseases by the support of traditional usage of herbal medicine indicates the therapeutic potential of *Evolvulus alsinoides* by its antimicrobial activity. Further clinical studies are required to validate the efficacy of this medicinal plant against various pathogens and various resistant strains of bacteria.

Keywords: *Evolvulus alsinoides*, HIV Secondary Pathogens, Solvent extracts, antimicrobial activity, Phytochemical Analysis.

INTRODUCTION

Human Immunodeficiency Virus (HIV) causes acquired immunodeficiency syndrome (AIDS) and a flurry of life-threatening opportunistic infections. Though this virus was discovered more than a decade ago there are no treatment methodologies available to date to completely cure this disease. HIV binds to CD4 receptors on helper T-lymphocytes and then the CD4 cells migrate to the lymphoid tissues. In the lymphoid tissues the virus gets replicated and then infects new CD4-positive cells. As the infection continues, depletion or impaired function of CD4 cells predisposes to the development of immune dysfunction. Globally Sexual transmission is the most common mode of transmission of HIV. Obviously, the probability of a person being infected via sexual intercourse depends on the likelihood of unprotected sex with an infected partner, so sexual behavior patterns are of major importance¹. In individuals with healthy immune systems, the symptoms of AIDS are primarily the result of conditions that mostly cause opportunistic infections that are normally controlled by the elements of the immune system which HIV damages.²

Antiretroviral drugs are now available to inhibit the replication of HIV. These drugs involved in restoring the patient's immune system to something approaching normal activity and reduce the chances of opportunistic infection developing to prolong life. Current antiretroviral drugs are vitally important to improve the quality and prolong the life of HIV/AIDS patients. However, these drugs have many disadvantages including resistance, toxicity, limited availability, high cost and lack of any curative effect. Thus, it is important to search for improved antiretroviral agents which can be added to or replace the current drugs in the anti-HIV armamentarium. Some plants are identified as an excellent source of anti-HIV agents. South Africa has a rich plant biodiversity and a long tradition of medicinal use of plants with approximately 3000 species of plants used as medicines.³ Preferred initial therapy is usually three drugs: efavirenz plus tenofovir or abacavir, plus lamivudine or emtricitabine. Other drugs are used in particular circumstances.⁴

Before 1990s, the problem of antimicrobial resistance was never taken to be such a threat to the management of infectious diseases. But gradually treatment failures were increasingly being seen in health care settings against first-line

drugs and second-line drugs or more. Recently the drug resistant nature of the microorganisms was increasingly to ensure their survival against the arsenal of antimicrobial agents to which they were being bombarded.⁵

There is a need to develop alternative agents for the control of pathogenic bacterial and fungal diseases in HIV infected persons. There is a good reason to support that the secondary metabolites of plants have evolved to protect them from attack by microbial pathogens. Hence, products from plants have great potential as sources of novel bioactive compounds for controlling pathogenic microorganisms. In general, plant-derived natural substances are considered as non-toxic and potentially effective against many pathogens. In recent years, interests have been generated in the development of safer antimicrobial agents such as plant-based essential oils and extracts to control many diseases.⁶

Nature has been a supply of therapeutic agents for thousands of years and a powerful range of contemporary medicine are derived from natural sources. Numerous active compounds derived from medicinal plants are assessed for the effectiveness and tolerability within the treatment of various diseases. Medicinal plants are identified and used to treat against various diseases in human history. Plants consist of several chemical compounds that are involved in various biological functions, together with defense against microorganisms like Bacteria, Virus, Fungi, Protozoa and saprozoic mammals. The chemical compounds in plants mediate their impact on the organic structure through processes the image of those already well understood for the chemical compounds in standard medicine; therefore, Herbal medicines don't disagree greatly from standard drugs in terms of how they work. Treatment with medicinal plants is taken into account terribly safe as there's no or lowest side effect. The Medicinal plants are an expensive resource of ingredients which might be employed in drug development either pharmacopoeial, non pharmacopoeial or artificial medicine.

Evolvulus alsinoides is a flowering plant belonging to the Convolvulaceae family. It is a natural equatorial distribution encompassing tropical and warm-temperate regions of India, Australia, Polynesia, Africa and also Americas. *Evolvulus alsinoides* could be a terribly slender, a lot of or less branched, spreading or ascending, typically extraordinarily bushy herb. The stems are 20-70 cm long and not twining. The leaves that are densely clothed with close, white and glossy hairs, are variable clothed, unsubdivided to ovate and frequently 0.5-1.0 cm in length; the apex is blunt with a tooth purpose and also the base is pointed. The flowers are pale blue and 6-8 millimeter in diameter. *Evolvulus alsinoides* has been found to contain Shankhpushpine.⁷ Evolvine (alkaloid, structure currently unknown). Evolvoids A and B which appear to be caffeic acid bound to two 2-methyl-1, 2, 3, 4-butanetetrol molecules and two caffeic acid molecules found to glucose (respectively).⁸ Evolvosides C, D, and E which are triglycosides of kaempferol. Evolvoside C (4'-O-β-d-glucopyranosyl-α-l-rhamnopyranosyl-β-d-glucopyranoside), D (O-methyl derivative of C), and E (di-O-methyl derivative of C).⁹ Other Kaempferol glycosides such as 4'-O-β-d-glucopyranosyl-β-d-glucopyranoside, 4'-O-α-l-rhamnopyranosyl-β-d-glucopyranoside, 7-di-O-β-d-glucopyranoside, 3-O-β-d-glucopyranosyl-β-d-glucopyranoside, and 3-O-β-d-glucopyranosyl-7-O-α-l-rhamnopyranoside.^{9,10} These bio-active compounds consist of both Antibacterial and Antifungal activity reported by the previous workers.

Vitexin and 4-methoxyvitexin.⁷ Quercetin as 3-O-β-glucopyranoside.^{8,11} Betaine (second hand reports).¹² Scopoletin¹³ and scopolin (coumarin and coumarin

glucoside).¹² Umbelliferone (coumarin) 2-methyl-1,2,3,4-butanetetrol, 1,3-di-O-caffeyl quinic acid methyl ester and caffeyl acid β-sitosterol, these compounds are identified by the GCMS and checked by their Antimicrobial activity against HIV Secondary pathogens.¹²

The present research works focus to; do develop new therapeutic agents or drug from selected medicinal plant of *Evolvulus alsinoides* against HIV Secondary pathogenic organisms, based on the review of literature, problems associated with treatment of pathogenic organisms and drug resistant patterns of the pathogenic organisms.

MATERIALS AND METHODS:

Sample Collection and processing:

The samples from secondary infection of HIV patients were collected from clinical laboratory at Government Hospital, Rasipuram. The specimens were transported immediately to the microbiology laboratory and processed without any delay. The samples were inoculated into the Nutrient Broth and incubated at 37°C for 24 hours, and also the samples were inoculated into Sabouraud Dextrose Broth and incubated at 25°C for 3 days.

After incubation the bacterial samples were inoculated on the Nutrient Agar medium and incubated at 37°C for 24 hours and also the fungal samples were inoculated into Sabouraud Dextrose Agar medium incubated at 25°C for 3 days. The organisms were subjected for further studies.

PREPARATION OF PLANT EXTRACT

Identification of plant materials

The plant authenticated by Botanical Survey of India (BSI-Southern Circle) - Government of India, Coimbatore, Tamil Nadu. Fresh leaves of *Evolvulus alsinoides* were collected from Kolli Hills, Tamilnadu and washed several times with water to remove the dust particles. The leaves of *Evolvulus alsinoides* were air dried at room temperature. Size reduction of plant material was done with the help of grinder to form powder.

Solvent selection

The following solvent Methanol, Ethanol, Petroleum ether and Chloroform was selected for extraction.

Extraction of *Evolvulus alsinoides* (leaves)

10 grams of *Evolvulus alsinoides* powder was used for extraction process. The extraction process was carried out by Maceration procedure. The 10gm *Evolvulus alsinoides* of with 100ml of all 4 solvents in beaker and kept in shaker at 70rpm for 3-4 days at room temperature.

PHYTOCHEMICAL ANALYSIS OF PLANT EXTRACTS:

Qualitative chemical tests were carried out using extract from plant to identify the phytochemicals. Preliminary phytochemical screening was carried out to find the presence of the active chemical constituents in extracts such as alkaloids, flavonoids, tannins, carbohydrates, phenolic compounds, terpenoids, glycosides, steroids, fixed oils and fats. In general, tests for the presence of phytochemical compounds involved the addition of appropriate chemical reagent(s) to the extract in test tubes.

The alkaloid was tested by using Mayer's test, the flavonoids were tested by lead acetate test. The tannins were tested by ferric chloride test. The total phenolic content in extract was determined by ferric chloride test. Steroids tested by salkowski test. The terpenoids tested by chloroform and con.sulphuric acid. The Carbohydrate was tested by benedict's test. The glycoside was tested by Aqueous NaOH.¹⁴

Antibacterial activity of *Evolvulus alsinoides* against isolated organisms

The antibacterial activity was carried out by well diffusion method. The sterile Muller Hinton agar plate was prepared. The isolated test organisms, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were spread over the Muller Hinton agar plates by using separate sterile cotton swabs.

The well was prepared with plant extract in 100 μ l concentration on Muller- Hinton agar plates with the following extracts, Petroleum ether, Chloroform, Ethanol, and Methanol. The plates were incubated for 24 hours at 37°C. The zone of inhibition of bacterial growth was measured.

Antifungal activity of *Evolvulus alsinoides* against isolated organisms

The antifungal activity was carried out by Well diffusion method. The sterile Muller Hinton agar plate was prepared. The isolated test organisms, *Aspergillus niger*, *Penicillium sp*, *Mucor sp* and *Candida albicans*, were spread over the Muller Hinton agar plates by using separate sterile cotton swabs.

The well was prepared with plant extract in 100 μ l concentration on Muller- Hinton agar plates with the following extracts, Petroleum ether, Chloroform, Ethanol, and Methanol. The plates were incubated for 2-4 days at 37°C. The zone of inhibition of fungal growth was measured.

RESULT AND DISCUSSION

Identification of isolated organisms

The isolated bacterial cultures from secondary infection of HIV patient's samples were identified as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* based on their Morphology, Staining, Motility and Biochemical test.

Table 1: Preliminary Phytochemical screening of various extracts of the leaves of *Evolvulus alsinoides*

Constituents	Ethanol	Methanol	Petroleum ether	Chloroform
Alkaloids	+	+	-	-
Flavonoids	+	+	+	-
Tannin	+	+	-	+
Carbohydrate	-	-	+	-
Terpenoids	+	+	-	-
Glycosides	+	+	-	-
Steroids	+	+	-	-
Phenols	+	+	-	+

+: Present - : Absent

Antibacterial activity of *Evolvulus alsinoides*

The antibacterial effects of Plant extract of *Evolvulus alsinoides* had been investigated against isolated *Escherichia coli*,

The isolated fungal cultures were identified as, *Aspergillus niger*, *Penicillium sp*, *Mucor sp*, and *Candida albicans*, based on their growth and LCB staining.

Identification of plant materials

The plant authenticated by Botanical Survey of India (BSI- Southern Circle) Government of India, Coimbatore, Tamil Nadu. A voucher specimen no is **BSI/SRC/5/23/2016/Tech: 278**, and the voucher was deposited at Department of Microbiology, MCAS, Rasipuram.

Phytochemical analysis of *Evolvulus alsinoides*

Phytochemical analysis of *Evolvulus alsinoides* was carried out by the following solvents Methanol, Ethanol, Petroleum ether and Chloroform.

In Methanol extract of *Evolvulus alsinoides* contain the components, Alkaloids, flavonoids, tannin, terpenoids, glycosides, phenolic compounds and steroids are present. Whereas the Ethanol extract of *Evolvulus alsinoides* contain the constituents, Alkaloids, flavonoids, tannin, terpenoids, glycosides, phenolic compounds and steroids are present. In Chloroform extract of *Evolvulus alsinoides* contain Tannin and phenolic compounds. Petroleum ether extract of *Evolvulus alsinoides* contains flavonoids, and Sugars are present. The predominant components present in all the extracts of *Evolvulus alsinoides* are Tannins, Phenols and Flavonoids (Table: 1)

The preliminary phytochemical screening carried out showed *Evolvulus alsinoides* contain some secondary metabolites such as glycosides, alkaloids, saponins, volatile oil, flavonoids and tannins. In general, secondary metabolites present in plants have been reported by Rabe.¹⁵ to be responsible for therapeutic activity. Singh & Bhat¹⁶ reported that flavonoids are responsible for the antimicrobial activity associated with some ethnomedicinal plants.

Table 2: Antibacterial activity of *Evolvulus alsinoides* extracts against isolated organisms

Organisms	Concentration of extract and zone of inhibition (mm)			
	Methanol extract (100 μ l)	Ethanol extract (100 μ l)	Chloroform extract (100 μ l)	Petroleum ether extract (100 μ l)
<i>Staphylococcus aureus</i>	03	03	01	05
<i>Klebsiella pneumoniae</i>	4.5	3.5	0.5	03
<i>E.coli</i>	08	3.5	5.5	5.5
<i>Pseudomonas aeruginosa</i>	-	-	-	-

Staphylococcus aureus, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. The well was prepared with plant extract in 100 μ l concentration and the wells were placed on Muller-Hinton agar plates (Table: 2)

The results clearly showed that Methanol, chloroform and Petroleum ether extracts were specific in action against the growth of bacteria. From this results, Methanol extract highly inhibit the growth of *E.coli* and *Klebsiella*. Whereas in Petroleum ether extract strongly inhibit the growth of *E.coli* and *S.aureus*

The similar investigation carried out by Kannan Elangovan *et al.*, (2013) the 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. Among all the extracts, aqueous showed higher activity followed by ethanol, ethyl acetate, chloroform and hexane.¹⁷

Antifungal activity of *Evolvulus alsinoides*

The antifungal effects of Plant extract of *Evolvulus alsinoides* had been investigated against isolated *Aspergillus niger*, *Penicillium sp*, *Mucor sp*, and *Candida albicans*. (Table: 3)

The well was prepared with plant extract in 100µl concentration and the well was placed on Muller-Hinton agar plates.

Table 3: Antifungal activity of *Evolvulus alsinoides* extracts against isolated organisms

Organisms	Concentration of extract and zone of inhibition (mm)			
	Methanol Extract (100µl)	Ethanol extract (100µl)	Chloroform Extract (100µl)	Petroleum ether Extract (100µl)
<i>Aspergillus niger</i>	02	1.5	1	1.5
<i>Penicillium sps</i>	1.5	01	-	02
<i>Mucor sps</i>	03	5.5	2.5	3.5
<i>Candida albicans</i>	04	01	-	02

The results clearly showed that ethanol, methanol, Chloroform and Petroleum ether extracts were specific in action against the growth of fungal culture. From the result, Methanol extract inhibit the growth of all fungal strains like *Aspergillus niger*, *Penicillium sps*, *Mucor sps* and *Candida albicans*.

Additionally, the ethanolic extract of the whole plant of *Evolvulus alsinoides* also showed the bactericidal activity against various clinical pathogens *including Staphylococcus aureus*, *Vibrio cholera*, *Salmonella thypi*.¹⁸ However, in another study, it was found that the ethanolic extract of *Evolvulus alsinoides* (whole plant) exhibited the antibacterial activity against *Pseudomonas aeruginosa* and *Escherichia coli* but found effective against *Staphylococcus aureus*, *Aspergillus niger* and *Candida albicans*.¹⁹

The presence of glycosides and alkaloids in *Evolvulus alsinoides* may be associated with their use by traditional medicine practitioners in healthcare systems in the treatment of fever, antibacterial and antifungal and venereal diseases. The results of this research highlight the fact that the methanol extracts exhibited greater antimicrobial activity because the antimicrobial principles. This inspection agrees with the report of other investigators of medicinal plants that organic solvents are more suitable for extraction of phytochemicals. The similar research work reported other researchers.^{20,21}

E. alsinoides (L.) L, exhibited antimicrobial properties against *Acinetobacter baumannii*, *Aspergillus niger*, *Cryptococcus neoformans* and *Candida albicans* and mild activity against *Bacillus subtilis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* when acetone extract was used, whereas methanolic extract is found to be highly effective.^{22,23}

CONCLUSION

There is a need to develop alternative agents for the control of pathogenic bacterial and fungal diseases in HIV infected persons. There is a good reason to support that the secondary metabolites of plants have evolved to protect them from attack by microbial pathogens. Hence, products from plants have great potential as sources of novel bioactive compounds for

controlling pathogenic microorganisms. In general, plant-derived natural substances are considered as non-toxic and potentially effective against many pathogens. In recent years, interests have been generated in the development of safer antimicrobial agents such as plant-based essential oils and extracts to control many diseases. The treatment of various infectious diseases by the support of traditional usage of herbal medicine indicates the therapeutic potential of *Evolvulus alsinoides* by its antimicrobial activity. Further clinical studies are required to validate the efficacy of this medicinal plant against various pathogens and various resistant strains of bacteria.

DECLARATIONS

Ethics approval and consent to participate

The permission to conduct this study was approved by the department of Microbiology, Muthayammal College of Arts and Sciences and the plant authenticated by Botanical Survey of India (BSI-Southern Circle) - Government of India, Coimbatore, Tamil Nadu. A voucher specimen no is BSI/SRC/5/23/2016/Tech: 278, and the voucher was deposited at Department of Microbiology, MCAS, Rasipuram.

Consent for publication: NA

Availability of data and materials: All data generated or analysed during this study are included in this published article

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