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Clinical Microbiology: Open Access

Research Article

Assessment of Phytochemicals and Antibacterial Activity of *Evolvulus alsinoides*

Hafiz Zaid Mahmood*

Institute of Microbiology, University of Agriculture Faisalabad, Pakistan

ABSTRACT

Objective: Utilization of plant-based medications for treating different diseases and individual health dates to the prehistoric ages. Plants and plant-based medicines are the premises of many advanced pharmaceuticals. In this research, the antibacterial properties of Slender Dwarf Morning–Glory (Evolvulus alsinoides) were assessed.

Method: 100 blood samples were collected, and Staphylococcus aureus was isolated and was confirmed by the biochemical tests. Molecular characterization was done by conserved sequences (16S rRNA gene) using Staphylococcus aureus integrated plasmid pUR3912 primers. Synthetic antibiotic Vancomycin was applied to check the antibacterial activity through Methicillin Sensitive Staphylococcus aureus. Antioxidant activity, Biochemical testing, Phytochemical analysis (—X-ray diffraction (XRD)‖ and —Scanning electron microscopy (SEM)‖ were used for better understanding and confirmation of phytochemical compounds extracted from Evolvulus alsinoides.

Result: Results showed that Methanolic plant extract has a clear antibacterial effect against Methicillin sensitive Staphylococcus aureus. Minimum inhibitory zone was 19 mm & 17 mm at 100 µg and 50 µg concentration respectively. On increasing concentration of plant extract minimum inhibitory zone also increase. Antioxidant activity of the methanolic extract was also proved by DPPH antioxidant assay *via* TLC.

Conclusion: This study can help in curing Methicillin sensitive Staphylococcus aureus infections with the use of Quercetin derivative which is a plant based flavonoid group of polyphenols extracts in future.

Keywords: Phytochemicals; Antibacterial; Medication

INTRODUCTION

Evolvulus alsinoides (E. alsinoides) also known as Slender dwarf morning-glory is a member of Kingdom Plantae, Order Solanales, Family Convolvulaceae and Genus Evolvulus. According to its morphology, it has diffuse, thick hair growth, a heavy growing herb with a woody fanned root-stock and many spreading branches discovered wild in many parts of South Asia in the fields and other tropical and subtropical areas. The plant is valuable in treating bronchitis, biliousness, epilepsy, leucoderma, increased hair growth, enhances appearance and hunger as per ongoing reports [1].

E. alsinoides is an exceptionally thin, pretty much stretched, spreading or climbing, normally to a great degree furry herb. Stem size is 20 to 70 centimeters (cm) in length, and not winding. Leaves are dressed and appressed thickly while white and plush hairs are variably dressed. Buds are light blue and 6-8 mm in distance across [2].

Convolvulaceae, referred to the ordinary group of blooming plants, incorporates 57 genera and around 1600 species. In Egypt, Convolvulaceae has 10 genera and 43 species. The family is broad in both tropical and subtropical regions. Convolvulaceae has numerous monetary uses as palatable crops, ornamentals, and therapeutic plants e.g., the sweet potato (Ipomoea batatas L.) and *Ipomoea aquatic Forssk.* It incorporates a few ornamentals utilized in agriculture, a few restorative plants (Ipomoea, Cuscuta, Dichondra, and Evolvulus) and various harmful weeds (Cuscuta and Convolvulus). Phytoconstituents of numerous types of this family incorporate; alkaloids, phenylpropanoids counting flavonoids, just as terpenoids and coumarins are constituents of plants of this family [3].

Ordinarily microbes assault, respiratory, GIT (Gastrointestinal) and urogenital tracts, skin and so forth. This prompts the utilization of therapeutic plants to treat diseases. New sources, particularly normal items from plants, are being explored because therapeutic

Correspondence to: Hafiz Zaid Mahmood, Institute of Microbiology, University of Agriculture Faisalabad, Pakistan; E-mail: MahmoodH@gmail.com

Received: February 05, 2021, **Accepted:** February 19, 2021, **Published:** February 26, 2021

Citation: Mahmood HZ (2021) Assessment of Phytochemicals and Antibacterial Activity of *Evolvulus alsinoides* . Clin Micro Biol.10:345.

plants have been generally utilized for the treatment of numerous sorts of intense and unending illnesses in Asia and numerous plants with antimicrobial action have been accounted for [4] (Figures 1 and 2).

Figure 1: Evolvulus alsinoides (Dwarf Morning- glory) in Talakona forest

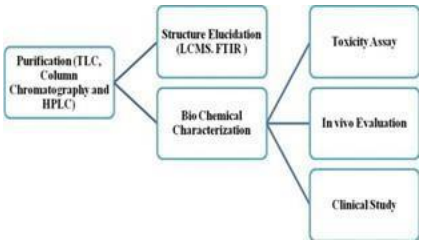

Figure 2: A brief summary of general approach for extraction and isolation of the bioactive compound.

There are unlimited medicinal potentials present in different plant extracts either pure plant or its extracts; all these abilities were due to the unmatched ability of different biochemical products obtained from these plants. Extraction is the most important step in isolation and characterization of the plant extract. For the analysis of the plant biochemical characteristics, the main methods they describe were HPLC and TLC as well.

WHO (World Health Organization) has recorded 21,000 plant types of the exploited world over for therapeutic reasons. In India, around 2,500 plant types having more than 1000 genera are being utilized in the indigenous arrangement of prescription. The WHO assessed that 80% popularity of nations depends on customary medicines, generally plant drugs. Despite colossal advancement in human drugs, irresistible ailments caused by microscopic organisms, growths, infections, and parasites are as yet a noteworthy risk to open wellbeing. The qualities of the plants that repress microorganisms and are imperative for human wellbeing have been looked into in research facilities since 1926.

Customary medicines, for which altogether plant materials are utilized, are named "Herbal medications". As indicated by the WHO, homegrown medication is characterized as plant determined constituents with curative or further human remedial advantages which have either simple or arranged ingredients from as a minimum one plant. In these, concoction parts (which are gotten from the plants or their subsidiaries), are utilized to produce the medications.

The extraction method mainly involves the separation of the medicinally active part of the plant from the inactive part. For separation of the active product, the solvent must be of the same polarity either polar or non-polar for actual separation. Basic parameters which can influence the effectiveness of the plant extract are plant parts used for the separation, the solvent used for separation, Technique used for the separation Effect of plant extract depends on the nature of the plant material, Origin, Degree of processing, Moisture content, Particle size [5].

A study was conducted to check the active compounds present in the magical plant Evolvulus alsinoides by using standard methods. Phytochemical screening revealed that there are steroids, triterpenes, alkaloids, phenols and flavonoids in the plant extract. The methanolic extract against methicillin and itraconazole has shown a positive effect against these and the negative control used was (DMSO). The medicinal effect of the plant was less for fungal stains then bacterial stains [6].

To check the biologically active compounds from plant material depend largely on the type of solvent used in solvent extraction. Properties of a good solvent in plant extractions include low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, inability to cause the extract too complex and not interfere with the bioassay [7].

In developing nations, an expansive extent of the populace (about 80%) is still relying upon customary medicinal frameworks for their essential human services. Besides, the offer of the medications, because of regular items in the worldwide market is about 30%. Because of promising restorative outcomes and the similarly low dimension of reactions, the interest for conventional home has grown prescriptions is persistently expanding all through the world including the created nations too. For instance, it has been assessed that the world market for natural medicines, including items and crude materials, has a yearly development rate of 5% to 15% [8].

World health organization (WHO) survey revealed that 20,000 medicinal plants are present in 91 countries including mega diverse living countries. The main steps involved in the utilization of biologically active reagents (biochemical) products from plant sources are extraction, isolation screening and characterization of the biochemical by-products of plants [9].

Research objectives

Isolation, identification and confirmation of Methicillin Sensitive Staphylococcus aureus at the molecular level

Discovering the bioactive synthetic constituents and to assess the antimicrobial action and examination of the concentrate of Evolvulus alsinoides

MATERIALS AND METHODS

Sample collection

Evolvulus plants were collected from roadsides of Lahore-Islamabad Motorway (M-2), near grassy cultivated areas. It was identified by its silent morphological feature a diffuse, thickly hispid, perpetual herb with a woody fanned root-stock and many spreading branches. The plant specimen was transferred to the processing lab under hygienic conditions. Then plants were washed twice with water and dried under shade for 1 week. After that it was ground into fine powder and its confirmation was confirmed by different techniques demonstrated in detail given below.

Extraction of constituents

Extracts were prepared in different solvents 70 % Methanol: Powdered plant extract i.e.100 grams was measured and mixed well in the ratio 1:3, then left at room temperature for 1 week. Repeated shaking with regular intervals was done. After blending the sample was then sifted through 3D channel paper and gathered in a different measuring utensil. Distilled Water: 10 gm of powdered plant extract and 100 ml of double-distilled water were mixed together and simmered for 15 min to make aqueous plant extracts. The Resulting extract was then sieved using Whatman's No.1 filter paper. After that purification was done by centrifugation at 6000 rpm for 20 min. Filtrate was collected into autoclaved vials to guarantee samples sterility and stored at 4 ℃ until for further use. Ethanol extract: For Ethanol solution, powdered plant extract was primarily defatted with petroleum benzene (60-80°C) followed by 1000 ml of ethanol through an extractor for 72 hrs at a temperature not beyond the solvent's boiling point.

Isolation of Crude Protein by SDS PAGE

100 mg plant powder was titrate in 1.5 ml TRIZOL reagent by persistent pipetting. For dissociation of nucleoprotein following steps were performed at room temperature for 2-5 mins. In these steps different standards were already pre-designed to identify specific crude proteins of the plant. 150 µl chloroform was added and test was permitted to remain at room temperature for 15 minutes. Then it was centrifuged at 13000 rpm for 15 minutes at 2-8°C. After centrifugation three layers were developed according to the gradient. Upper dry stage comprising RNA, Lower red layer was wanted protein, while interphase had genomic DNA. Water was sucked persistently through pipette. For protein extraction 450 µl of 100 % ethanol was used. 2 ml of 2-propanol was added to isolate the protein. Test was continued for 10 minutes at room temperature. Centrifugation was done at 13000 rpm for 10 minutes at 2-8°C. Supernatant was discarded. Pellet was washed with 2 ml of 95 % ethanol. Each wash stayed for 20 minutes. Centrifuged at 9000 RPM for 5 minutes at 28°C.After washing 3 ml of 100 % ethanol was added and vortex pellet. Furthermore, 20 minutes centrifugation at 9000 rpm for 2-8 °C. Protein pellet was dried and broken down in 1 % SDSwith constant pipetting. Insoluble material was discarded by centrifugation at 12,000 rpm for 10 minutes. Supernatant was transferred to another cylinder. Phytochemical analysis of plant matter was done through X-ray diffraction (XRD). XRD was carried out to examine the crystallographic structure of the plant matter& molecular composition.

Biochemical characterization of bacteria

To make differentiation among Gram positive and negative bacteria this test is used in which a glass slide droplet of water was taken. Then single separated bacterial colony was mixed for smear formation. For staining Crystal violet dye was added on slide for 2 minutes and then Gram's iodine was added on smear to make complex between peptidoglycan and principal dye for 01 minutes. After washing acetone (decolorizer) was use for 1 minute. Safranin was applied as counter stain. Then smear was washed, marked and inspected under microscope at 100X.

RESULTS

Staphylococcus aureus isolated was then tested against Vancomycinby disc diffusion method. Results showed that staphylococcus aureusis sensitive to Vancomycin as shown in .03.01. For confirmation of MSSA effect of different antibiotics like Piperacillin, Meropenum, Amikacin, Tobramycin, Amoxicillin, Imipenum, and Cefoxitin were used to check growth of Staphylococcus aureus against these antibiotics. In.03.02 and 03.02 results showed that Staphylococcus aureus is sensitive against Amikacin, Tobramycin and Amoxicillin. On blood agar disc diffusion method was performed and results showed that staphylococcus aureus isolates had clear sensitivity against Vancomycin.

DISCUSSION

Rising anti-toxins and scarcity of more recent antimicrobials encourage the researcher to elaborate on different natural extracts to be used as an antimicrobial effect [10].An essential endeavor is the requirement for a novel, incredible and eases medicate medicines to treat microbial ailments, particularly in emergent nations of the world, where the main culprit nature is theirritable disorders caused by the misuse of the antibiotics, because of which resistance developed in the body against antibiotics[11].

In Asia, therapeutic plants are widely used by all people of age, both in people and in people taking pills in exceptional native drug structures like Siddha, Ayurveda and Unani and indirectly in courses of pharmaceutical action. Previous work has found that Gram-negative microorganisms are generally increasingly safer compared to Gram-positive microorganisms. The oil of some fragrant plants with a complex level of carvacrol and thymol has a greater capacity against the bacterial strain. Another similar study was conducted by Handayani[12]. Antibacterial activity of polyphenol compounds has been investigated against different concentrations of Staphylococcus aureus and *Escherichia coli*. The Agar disk diffusion method was used to measure the inhibition zone. Results demonstrated that *Escherichia coli* have no bacterial activity while Staphylococcus aureus had bacterial activity at various concentrations of the plant extract, which is Methanolic. Statistic tested was done by one way ANOVA and it was shown that Staphylococcus aureus had significant differences at each concentration from polyeugenol[13].

Another similar study was done by Cheyma [14] December. The main objective of the study was to find out naturaland free ofside effect compounds for various bacterial strains. For the study crude methanol extracts of Palm *(Phoenix dactylifera L)* fruits (n=5) were taken and tested for antibacterial action against bacterial strains. Results demonstrated that methanol extracts showed good antibacterialactivityagainstallisolates *Pseudomonas aeruginosa (P. aeruginosa), Escherichia coli (E. coli)* and Staphylococcus *aureus (S. aureus).* But most sensitive was *E. coli* showed high sensitivity to the Gh extract with inhibition value 13.667 mm, while P. aeruginosa showed high sensitivity to the DB extract with inhibition value 10.667 mm and *S. aureus* showed high sensitivity to this extract with inhibition value 13.000 mm. Similarly the AgNPs from Leucas aspera flower showed 2.30 mm and 2.11 mm ZoI against test organisms: *S. aureus* and *Bacillus* subtilis respectively [15].

In Present study, 100 contaminated blood samples were collected from different hospitals of Lahore and Methicillin Sensitive *S. aureus* was isolated from them using a well plate method of Balouiriet et al, (2016) [16]. Biochemical tests for confirmation of staphylococcus aureus and plant extract were performed. After

that Synthetic antibiotic Vancomycin was applied to check the antibacterial activity MSSA and it shows sensitivity to Vancomycin. Molecular categorization of Isolated S. aureus was also done by using PCR conserved sequence (16SrRNA) with universal primers. For antioxidant activity and phytochemical analysis technique used was X-ray diffraction (XRD). It was deployed for better understanding and confirmation of biochemical compounds extracted from Evolvulus alsinoides. In XRD the hypothetical size of bioactive mixes was inspected. Quercetin derivatives appeared as major component, belong to the Flavonoids class, which are valuable as antibacterial and antioxidant. In future, these bioactive mixes can be used to discover the correct measure of these mixes in the 01 mg of plant for pharmaceutical purposes. Alternate types of E. alsinoides demonstrated the antimicrobial action because of the nearness of monoterpenes or flavonoids [17].Also, the nearness of phenylpropanoids, coumarins, flavonoids and triterpenes in *Evolvulus alsinoides* herb appeared [18].

To assess the antioxidant potential of diverse plant extract and methanolic extracts, scavenging agent (DPPH 2, 2 –diphenyl-1-picrylhydrazyl) was used. For this purpose diverse concentrations of extracts were spotted on the TLC plate. After drying, 0.2% of DPPH solution was sprayed on a TLC plate if the yellow color appeared on the spot it meant that plant extract had antioxidant activity.

CONCLUSION

Based on the results, it was concluded that E. alsinoides can play an essential role in the treatment of infections caused by methicillin-sensitive *Staphylococcus aureus*. In developing countries like Pakistan herbal medicine has great scope and can be effectively utilized as against different bacterial infections and can overcome development of antibacterial resistance in human beings.

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