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Identification and evaluation of antimicrobial properties in the extract of Vallaris Solanacea

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Abstract

The emergence and spread of multidrug resistant bacterial pathogens have substantially threatened the current antibacterial therapy, warranting the search for other alternatives. New antibacterial treatments are urgently needed. India is one of the twelve mega biodiversity centers having more than 45,000 plant species. A vast ethnobotanical lore exists in India from ancient time which can be of real use in the formulation of effective antimicrobials. Herbal world is continuously explored for natural therapeutics. In this paper a discussion is made on antimicrobial properties of plants. Evaluation of antibacterial properties of *Vallaris solanacea* was done. Anti bacterial activity of methanolic extracts of *Vallaris solanacea* was screened by agar disc diffusion method. The evaluated plant extract was found to exert a range of in vitro growth inhibitory action against the tested bacterial species, namely gram positive bacteria (*Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli*). Plant extract displayed a potential antibacterial activity with MIC 100.0 µg/ml. To understand the mechanism of antimicrobial action, inhibition analysis of plant extract with para chloro mercury benzoate showed it was a cysteine protease.

Keywords: Vallaris solanacea, natural therapeutics, antimicrobicidal.

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Introduction

The spread of multiple antibiotic resistant pathogenic bacteria has been recognized by the World Organization for Animal Health (OIE), the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) as a serious global human and animal health problem. To solve this problem, plants form a good solution. Plants are a goldmine of novel chemicals; many modern drugs has been developed from them. Many of them have been valued for their antimicrobial effects and medicinal powers in addition to their flavor and fragrance qualities [1-5]. There are more than 2,70,000 higher plants existing on this planet. But so far less than 10% of recorded flora has been explored phytochemically and evaluated for various biological activities [6]. While vast majority of the plant resource is waiting for discovery, these plant based traditional medical systems continue to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care [7]. The world is now looking towards India for new drugs to manage various challenging diseases due to its rich biodiversity of medicinal plants and abundance of traditional knowledge such as Siddha, Ayurveda and Unani [8]. The antimicrobial plant extracts extend the shelf life of foods mainly because of their preservation action against microorganism. The spread of multiple antibiotic resistant pathogenic bacteria has been recognized by the World Organization for Animal Health (OIE), the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) as a serious global human and animal health problem.

Traditionally used medicinal plants have recently attracted the attention of the biological scientific communities. The antibacterial activity of plant extracts can be attributed not only to a single bioactive principle but also in concert action with other compounds. It is estimated that only one percent of 2,65,000 flowering plants on earth have been studied exhaustively for their chemical composition and medicinal value [9].Today, nearly

88% of the global populations turn to plant derived medicines as their first line of defense for maintaining health and combacting diseases. One hundred and nineteen secondary plant metabolites derived from plants are used globally as drugs; 15% of all angiosperms have been investigated chemically and of that 74% of pharmacologically active plant derived components were discovered. In the present study we made an attempt to purify, evaluate antimicrobial properties of plants extract Vallaris Solanacea belonging to family Apocyanacea., green synthesis of nanoparticles , silver nanoparticles being used as antimicrobial agents, combination therapy etc. The antimicrobial properties of plants and plant derived nanoparticles were studied. A combination therapy to combat the antibiotic resistance was also studied.

Methodology

Many plants have been screened for antimicrobial activities in the Botanical Gardens of Bangalore, Zoo park at Tirupathi, Biodiversity Park at Visakhapatnam and herbariums in different places of which *Vallaris solanacea* was a novel plant and was selected to check for its antimicrobial properties.

Determination of Antimicrobial activity of the crude extracts of *Vallaris solanacea*:

Plant product preparation *Vallaris solanacea* stems were obtained from Biodiversity Park at Visakhapatnam. Disease free leaves were collected. The product was powdered and extracted in suitable solvent. The collected leaves were surface sterilized with 0.1% mercuric chloride and then washed with D/W 2-3 times separately & shade dried. Fine powder were made after complete drying and used for the experimental work. The product was powdered and extracted in suitable solvent. The sample was then filtered through Whatmann No. 1 filter paper and concentrated to dryness under reduced pressure using a rotary evaporator to obtain crude extracts. The aqueous extracts were dried under reduced pressure by means of a freeze-drier. Crude extracts were then stored in the dark at 10^{o}C until use. Fine powder were made after complete drying and used for the experimental work.

Solvent Extraction of Leaves

Extracts were made in 80% methanol at room temperature by simple extraction method. 10 gm dried powder of leaves mixed with 100ml solvent in 250 ml flask and were kept on shaker for 24 hrs. Then it was allowed to stand for the 30 min to stand the plant material. Thereafter it was filtered and centrifuged at 5000 rpm for 15 min .The supernatant was collected &solvent was evaporated at 45 0C in vacuum evaporator to make the final volume 1/5 of the original volume. Different concentrations of plant extract namely 10 - 200µg/ml of plant extract was used for the experiment and 100µg/ml was the minimum inhibitory concentration for *Escherichia coli* and *Staphylococcus aureus*. The MIC concentration was standardized and that concentration was used for further experiments.

Determination of antimicrobial activity

Culture media for antibacterial test Nutrient Agar/broth was poured into suitable plates. Inoculums Preparation, The bacteria were inoculated into Nutrient broth and incubated at37^{o}C for 24 hrs.

Microorganism Used

Cultures of Staphylococcus aureus and Escherichia coli against which the antibacterial activity of plant extract was to be determined. The Antibacterial activity of methanolic extract analysed by using disc diffusion assay sterile disc was used for the present investigation. Antimicrobial activity of the crude extracts was determined against microbial stains by agar well diffusion method. Gentamycin was used as standard drug for comparison of antimicrobial activity against bacteria. The zone of inhibition was compared with standard drug after 24h of incubation of bacteria. Control is also maintained.

Disc Diffusion Assays

The antibacterial activity of methanolic extract of plant was analysed by using disc diffusion assay. Sterile disc dipped in plant extract was used for the present investigation. Gentamycin was used as standard drug for comparison of antimicrobial activity against bacteria. The zone of inhibition was compared with standard drug after 24h of incubation of bacteria at 37C. Control is also maintained separately comprising inoculums without plant extract. Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were recorded.

Turbidometric Assays

The effect of plant extract was examined on the growth of *Escherichia coli* and *Staphylococcus aureus* in liquid culture. When bacteria are grown in liquid medium, the medium starts off clear and ends up being cloudy, or “turbid”. The plant extract stopped the bacterial culture from going cloudy,

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showing that it stopped bacterial growth. The cloudiness or turbidity of the bacterial culture can be measured using a spectrophotomer.

Inhibition analysis

This analysis involves specific protease inhibitors to identify catalytic groups in the active centre of protease. Inhibitors are important in the study of proteases; they give the clearest evidence as to type catalytic site, information that forms the basis of classification of enzymes. This is to compare the mechanism of antimicrobial action of *Vallaris solanacea* with that of other proteases.

Evaluation of the synergistic effect of antibiotics and plant extract on resistant bacterial samples

This evaluation was done according to Muroi and Kubo [10]. Aliquots of 100 µL of resistant bacterial cultures (106 cells/ mL) grown in 10 mL of nutrient broth for 6 h were inoculated in nutrient broth supplemented with the respective antibiotics (50 µg/mL) with different concentrations of plant extracts. The concentration for plant extract ranged from 10 to 200 µg/mL, based on MIC values, that had previously been evaluated. After 48 h, the optical density of each sample was documented to verify any synergistic effect among the tested compounds. However, a synergetic effect was observed when 50 µg/mL was combined with each one of the antibiotic tested, even those that did not show any activity by themselves.

Results

Anti bacterial activity of methanolic extracts of *Vallaris solanacea* was screened by agar disc diffusion method. The evaluated plants extract was found to exert a range of in vitro growth inhibitory action against the tested bacterial species, namely gram positive bacteria(*Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli*). Plant extract displayed a potential antibacterial activity with MIC 100.0 µg/ml. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant pathogens. The main antimicrobial effect of allicin in garlic is due to its chemical reaction with thiol groups of various enzymes, e.g. alcohol dehydrogenase, thioredoxin reductase, and RNA polymerase, which can affect essential metabolism of cysteine proteinase activity involved in the virulence of *E. histolytica*. Inhibition analysis, involve specific protease inhibitors to identify catalytic groups in the active centre of protease. Inhibitors are important in the study of proteases; they give the clearest evidence as to the type catalytic site, information that forms the basis of classification of enzymes. Using inhibition analysis, *Vallaris solanacea* was found to be a cystein protease and supported the mechanism of antimicrobial effect of allicin in garlic. Inhibition of certain thiol containing enzymes in the microorganisms by the rapid reaction of thiosulfinates with thiol groups was assumed to be the main mechanism involved in the antibiotic effect [10]. The result indicates the potential usefulness of the plant in treating microbial infections in humans and justifies the need for further investigations and characterization of the bioactive compounds present in the methanolic extracts of the plants.

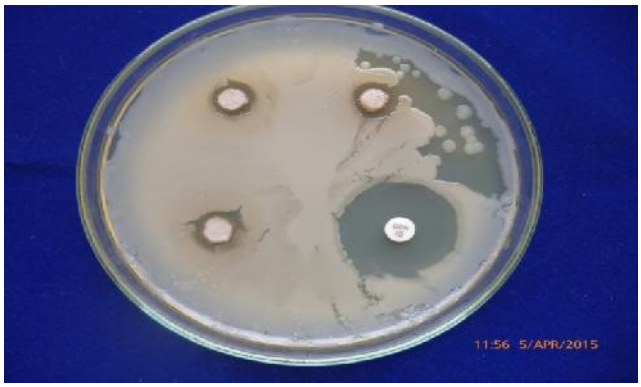

Figure 1: Antimicrobial activity of the crude extracts of *Vallaris solanacea* against E.coli

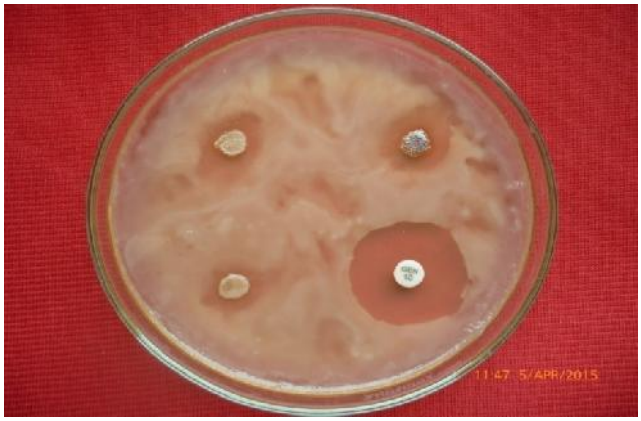

Figure 2: Antimicrobial activity of the crude extracts of *Vallaris solanacea* against Staphylococcus aureus

The antibacterial activity of methanolic extract of Vallaris solanacea was analysed by using disc diffusion assay sterile disc was used for the present investigation. Antimicrobial activity of the crude extracts was determined against microbial stain (E.coli) by agar well diffusion method. Gentamycin was used as standard drug for comparison of antimicrobial activity against bacteria. The zone of

inhibition was compared with standard drug after 24h of incubation of bacteria [See figure 1 and figure2].

Phyto chemicals	Mechanism
Phenolics	Membrane disruption, substrate deprivation
Phenolic acids	Bind to adhesins, complex with cell wall, inactivate enzymes
Terpenoids, essential oils	Membrane disruption
Alkaloids	Intercalate into cell wall
Tannins	Bind to proteins, enzyme inhibition, substrate deprivation
Flavonoids	Bind to adhesins, complex with cell wall, Inactivate enzymes
Coumarins	Interaction with eucaryotic DNA
Lectins and polypeptides	Form disulfide bridges

Table 1: Shows Mode of action of phyto chemicals

Green synthesis of silver nanoparticles

It is a well-known fact that silver ions and silver-based compounds are highly toxic to microorganisms which include 16 major species of bacteria [11, 12]. Silver nanoparticles are being used as antimicrobial agents in many public places such as railway stations and elevators in China, and they are said to show good antimicrobial action.The major advantage of using plant extracts for silver nanoparticle synthesis is that they are easily available, safe, and nontoxic in most cases, have a broad variety of metabolites that can aid in the reduction of silver ions, and are quicker than microbes in the synthesis. It was suggested that the phytochemicals are involved directly in the reduction of the ions and formation of silver nanoparticles [13]. Some of the silver-synthesizing plants include Medicago sativa, Azadirachta indica, Aloe vera, Cinnamomum camphora leaf, Carica papaya fruit, Cinnamomum zeylanicum bark, Jatropha curcas etc.

With the advent of safer silver nanoparticle based wound dressings there can be a reduction in antibiotic resistance. Silver nanoparticles are used in bone cements that are used as artificial joint replacements. Polymethyl methacrylate loaded with nanosilver is being considered as bone cement. Nanosilver can be utilized for detecting various abnormalities and diseases in the human body including cancer [14]. The plasmonic properties of nanosilver also make it an excellent candidate for bioimaging as they, contrary to commonly used fluorescent dyes, do not undergo photobleaching and can be used to monitor dynamic events over an extended period of time [15].

Combination therapy

Antibiotics are traditionally defined as natural compounds, produced by microorganisms, with selective antibacterial activity that does not have any strong side effects on human. Their mechanism of action is either through killing the bacteria (bactericidal effect) or by inhibiting bacterial growth (bacteriostatic effect). The discovery of antibiotics had eradicated the infections that once ravaged humankind. But their indiscriminate use has led to the development of multidrug-resistant pathogens. Around 90–95% of *Staphylococcus aureus* strains worldwide are resistant to penicillin [16] and in most of the Asian countries 70–80% of the same strains are methicillin resistant [17].

An alternative approach is the use of combination therapy i.e. synergism between known antimicrobial agents (antibiotics) and bioactive plant extracts. Combination therapy or synergistic therapy; against resistant microorganisms may lead to new ways of treating infectious diseases and probably this represents a potential area for further investigations. Combination therapy is helpful and useful for patients with serious infections caused by drug resistant pathogens. The mode of action of combination therapy significantly differs from that of the same drugs acting individually; therefore the selection of an appropriate combination is crucial and essential which requires understanding the potential interaction between the plant extracts and antimicrobial agents. Souto de Oliveira et al. [19] investigated the synergistic activity of norfloxacin, tetracycline and erythromycin with ethanol extract of *Mangifera indica* L. peel against *S. aureus* strains. The study indicated that mango peel could serve as a source of potential adjuvant of antibiotics, which adds value to this mango by-product.

Discussion

The antibacterial activity of methanolic extract of *Vallaris solanacea* was analysed by using disc diffusion assay. It is estimated that only one percent of 2,65,000 flowering plants on earth have been studied exhaustively for their chemical composition and medicinal value [9].Today, nearly 88% of the global populations turn to plant derived medicines as their first line of defense for

maintaining health and combating diseases. One hundred and nineteen secondary plant metabolites derived from plants are used globally as drugs; 15% of all angiosperms have been investigated chemically and of that 74% of pharmacologically active plant derived components were discovered. The search for antimicrobials from natural sources has received much attention and efforts have been put in to identify compounds that can act as suitable antimicrobials agent to replace synthetic ones. Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines in controlling the growth of microorganisms. These compounds have significant therapeutic application against human pathogens including bacteria, fungi or virus. Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds. Therefore, medicinal plants are finding their way into pharmaceuticals, neutralceuticals and food supplements. Plants form cheap, safe and easily available source of pharmaceuticals with very neglegible or no side effects. The plant extracts generally show proteolytic activities, thus considered proteases. Several plant families such as Asteraceae, Caricaceae, Moraceae, Asclepiadaceae, Apocynaceae and Euphorbiaceae as the protease bearing plant families. Particularly plant proteases are active over wide range of pH and temperature. Latex is a milky exudates formed from the leaves of plants. Plant latex is also considered as natural source of pharmaceuticals and pesticides. Combination therapy and plant derived nano particles would be the best ways in combating the antibiotic resistance in microorganisms and pave a novel path for new pharmaceuticals. The knowledge of plants used by traditional herbal healers for ailments would be immense help to replace synthetic drugs.

Conclusion

From the present study, it can be concluded that the traditional plants may represent new sources of antimicrobials with stable, biologically active components that can establish a scientific base for the use of plants in modern medicine. The result indicates the potential usefulness of the plant extract of *Vallaris solanacea* in treating microbial infections in humans and justifies the need for further investigations and characterization of the bioactive compounds present in the methanolic extracts of the plants.

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