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EVALUATION OF INVITRO ANTIMICROBIAL ACTIVITY OF *HIBISCUS HIRTUS LINN*

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ABSTRACT

The present study is initiated to discover the antibacterial potential of the sub shrub *Hibiscus hirtus L.* The leaves of this traditional shrub were found to posses' antibacterial activity, which were extracted using methanol as solvent and soxhlation as a method of extraction. The leaves were priorly investigated for their pharmacognostic, phytochemical amd physical parameters. The antibacterial activity of the methanolic leaf extract was carried at different concentrations on strains of gram positive and gram negative bacteria such as Staphylococcus aureus and Escherechia coli respectively by using agar well diffusion method or cup and plate method. The results were furnished as the zone of inbition obtained around each concentration of the extract and were compared with that of standard antibiotic used in the experiment, streptomycin.

KEYWORDS: Antimicrobial activity, agar well diffusion method, methanolic leaf extract, pharmacognostic, phytochemical parameters.

INTRODUCTION

Plants have been utilized as therapeutic agents since the time immemorial in both organized and unorganized forms. Plant, animal and mineral products were the ultimate source of treatment. Plants produce a large number of components which act as averting agents, helping them protect from microbial infection and deterioration.[1] Different substances have been identified in many medicinal plants and shrubs which are believed to be the antimicrobial agent, and these include different forms of alkaloids, diterpenes, saponins, flavanoids, sterols, quinines, different forms of other proteins and lipids as well. Many efforts

have been laid down to discover new antimicrobial compounds from various kinds of origins. Systematic screening of them may result in exploration of new effective compounds.[2]

In developing countries, emerging synthetic drugs are not only unaffordable and inadequate for the treatment of diseases but are also often devalued and associated with many side effects.[3,4] In the past three decades, pharmacological industries produced many number of novel antibiotics. However these antibiotics have failed to trouble the growth of many bacteria that have genetic capability to transmit and acquire resistance to drugs. Thus, diseases caused by these bacteria lead to high mortality and morbidity peculiarly in the case of immune-compromised subjects.[5]

In addition, many researchers have investigated and established the side effect of misuse and overuse of antibiotics which have the potential to harm integral organs of the body such as kidney, liver and some cells of pancreas and spleen as well as a huge impact on immune system. The successful outcome of traditional medicine has ushered the search for modernistic chemotherapeutic substitutes to phase out the infection caused by drug-resistant microbes and to scale down the damage caused by the antibiotic.[6] However, plants have an almost perpetual ability to produce compounds that have distinct bioactive principles that we can't synthesize. The healing property of many herbal medicines has been recognized in many ancient cultures.[7]

On an epitome, large number of plant species have not been studied for potential medicinal value or described for it but various studies have been published, investigating the anti-fungal and anti-microbial activities of plant derived compounds against a range of pathogens.[8] So far, no report proving the anti-microbial activity of the leaves of plant *Hibiscus hirtus L* have been established. Therefore, the present study focuses at evaluation of anti-bacterial activity of leaves of *Hibiscus hirtus L* in experimental models.

Hibiscus hirtus L belongs to family Malvaceae commonly known as lesser mallow is a small tropical sub shrub or perennial of genus Hibiscus which can grow up to 2ft. It is a diminutive species from the East Indies and Malaysia. It is a traditional shrub that has its own importance in medicine.[9]

Classification[10]

Kingdom : Plantae

Division : Tracheophyta

Class : Magnoliopsidae

Order : Malvales

Family : Malvaceae

Sub family: Malvoideae

Genus : Hibiscus

Species : *Hibiscus hirtus L.*

EXPERIMENTAL DESIGN

Procurement of plant material

For the present experimental analysis, *Hibiscus hirtus. L* leaves were collected in the month of September 2018 from Sircilla village of the Karimnagar district. The plant was identified and authenticated by BSI/DRC/2018-2019/TECH/673. The leaves were dried in shade and stored at 25°C. It was powdered, passed through sieve no.40, and stored in air-tight container.

Drugs and Chemicals

Streptomycin (Nitin life sciences limited), Methanol (Merck Life Sciences [p] Ltd.), Agar medium (Hi-Media laboratories Pvt. Ltd), and Broth (Central Drug House Pvt. Ltd) were used during the experiment.

ANTIBACTERIAL EVALUATION[11,12]

Preparation of extracts

Methnolic extract of the leaves of *Hibiscus hirtus.L* was prepared by using soxhlation as the method of extraction at a suitable temperature of 55°C. 50 g of powdered leaves is prepared as a thimble and placed in the condenser and placed it in the round-bottomed flask in which required amount of methanol was taken. Soxhlation process was carried out for 6-8 hrs. The extract obtained was evaporated and dried in desiccators.

Selection of bacterial strains

Clinically and medicinally important bacterial strains used in the investigation were *Staphylococcus aureus* and *Escherichia coli* which were considered as test pathogens in the experiment.

Review on test organism

Staphylococcus aureus is very important pathogen (gram positive) that causes a variety of diseases including skin infections. Gastro intestinal disease (*staph*) food poisoning, toxic shock syndrome and nosocomial infections acquired during hospitalization.

Escherichia coli are bacteria (gram negative) that exists naturally as part of normal gut of healthy humans and mammals. It causes enteric diseases. However, there are relatively few strains of this organism, which are pathogenic to humans and are associated with food related illness

Standard reference antibiotic

The reference antibiotic used is streptomycin acquired from Hi-media.

Preparation of Broth Culture

The liquid broth culture which acts as a nutrient media for the bacteria is prepared according to standard composition for broth culture media. Sterilization in autoclave was performed in order to make media sterile from viable micro organisms and spores. After the sterilization of media, the bacterial strains were inoculated under laminar air flow. The incubation of inoculated media was carried out at 37°C for 48 h.

Preparation and Sterilization of Nutrient media

The required amount of nutrient agar was weighed and dissolved in 500 ml of distilled water and its dissolution is enhanced by heating with stirring continuously on a hot plate then plug with cotton and autoclave for 15 min (121°C, 15 lbs pressure) in order to sterilize the media.

Media plating

Sterilized media was poured into the Petri plate (presterilized in oven for 2 h at 200°C to avoid any possible extraneous contamination). The plated Petri dishes were kept on a flat platform to avoid non-uniform solidification of medium. All these operations were done in a sterile room which was contoured with laminar air flow supported with HEPA filters.

Bacterial culture preparation

Bacterial cultures were inoculated in the freshly prepared nutrient broth (which was prepared and sterilized priorly) and kept on rotary shaker for 24 hours and observed for growth (turbidity indicates the growth). Overnight old cultures are used for testing and determination of each extract.

ANTIMICROBIAL ASSAY

Agar well diffusion method

Agar well diffusion technique is mainly used to ascertain the antimicrobial activity of plants or microbial concentrates. In essence to the approach utilized as a part of disc diffusion technique, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the whole agar surface. At that point, openings with a width of 6–8 mm are punched aseptically with a sterile cork borer or a tip, and a volume (150 µl) of the antimicrobial agent or extract solution at desired concentration (40 mg/ml, 50 mg/ml) was poured in the well. A negative control was maintained using 150 µl of sterile water in a well and 150 µl of standard antibiotic (streptomycin of 10 mg/ml) was the positive control, were also introduced into the well. Duplicates were maintained for each extract. Then, agar is incubated for 18–24 h at 37°C under sterile conditions depending on the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested.

RESULTS

By the present results, it was ratified that the leaf extract of *Hibiscus hirtus L* has antimicrobial activity. The different concentration of methanolic extract by soxhlation process shows antimicrobial activity against the tested microorganisms *Staphylococcus aureus* and *Escherichia coli*. The objectives of the present paper were to study antimicrobial activity of methanolic extracts of the leaves of *Hibiscus hirtus L*. The methanolic extract showed minimum inhibitory concentration (MIC) against all the organisms at concentration (40 mg/ml, 50 mg/ml) as shown in Table 1. Methanol extract was effective against *Staphylococcus aureus* and *Escherichia coli* with MIC being 150 µl. The extract obtained from soxhlation formed zone of inhibition of which the plate inoculated with Staphylococcus aureus (40mg/ml) expressed a zone of inhibition of 24mm and that with Escherichia coli showed 11mm antibacterial zone of inhibition.

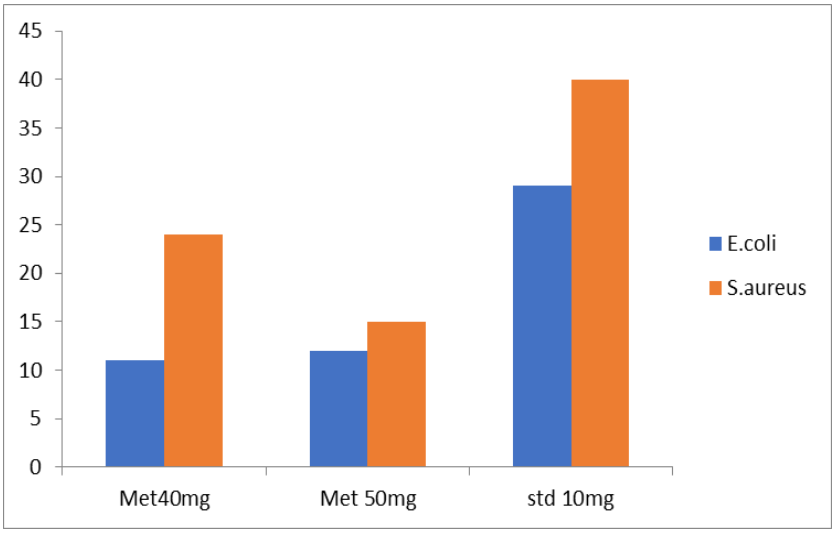
By results it is concluded that the extract did not show dose dependant antimicrobial activity. The zone of inhibition against *Staphylococcus aureus* was higher when compared to that with the one with *Escherichia colI.*

Table 1: Antibacterial activity of methanolic extracts of leaves of *Hibiscus hirtus L*.

Type of micro organism used	Diameter of zone of inhibition		Standard (streptomycin)
	Methanol extract concentration		
	40mg	50mg	10mg
Escherichia coli	11mm	12mm	29mm
Staphylococcus aureus	24mm	15mm	40mm

Staphylococcus aureus **Escherichia coli**

Antibacterial Activity



DISCUSSION

Plants are prospective source of antimicrobial agents in different countries. Plants are rich in a variety of phytochemicals including tannins, terpenoids, alkaloids, and flavonoids which have been found in vitro to have antimicrobial properties. Although the mechanism of action and efficacy of these herbal extracts in most cases is still needed to be validated scientifically, these preparations mediate important host responses.

The extract here did not inhibit Escherichia coli to a greater extent because it is a gram negative type of bacteria and it contains a firm layer of peptidoglycan chain with upto hundred disaccharide units in it which do not allow the anti-microbial agent to perfuse through it. This might be considered as the solid evidence for the lesser potent activity of the present methanolic extract against *Escherichia coli*.

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