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Anti-Bacterial and Anti-Fungal Action of Crude Solvent Extracts of *Soymida Febrifuga*

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ABSTRACT

The antimicrobial and antifungal activities of various solvent extracts of Soymida febrifuga were determined against a wide variety of pathogenic bacteria and fungus. Crude extracts of Soymida febrifuga shows mild to moderate activities for most of the treated bacteria and almost no effect on tested fungal species except two. Ethyl acetate extract shows good activity against tested bacteria and fungus, n-hexane extract and methanol extract showed anti- bacterial effect against only of the two tested organisms. It has been expected that the present work on antimicrobial and anti-fungal screening of the plant material will help scientists who want to do their work in designing clinical drugs concerning the killer diseases.

Keywords: Meliaceae, Soymida febrifuga, antibacterial, antifungal.

INTRODUCTION

The tribal folks spread across the country make use of medicinal plants through oral traditions. In India several thousands of plant species are being used by thousands of ethnic communities [1]. *Soymida febrifuga* commonly known as mamsarhohini of family meliaceae, is a reputed folk medicinal plant [2]. Soymida febrifuga sometimes called Swietenia febrifuga and in telugu cevamanu, cheramaanu, Sommi. The decoction of the bark contains a resinous bitter principle well adapted for gargles, vaginal infections, enemata, rheumatic swellings, and stomach pain. The bark is said to be used as an anti-cancer remedy [3], for blood coagulation, wounds, dental diseases, uterine bleeding and haemorrhage [4] and acrid, refrigerant, antihelminthic, aphrodisiac, laxative; good for sore throat; removes "vata"; cures 'tridosha' fevers, cough, asthma [5] and is anti-inflammatory [6] in action. It is used as an important drug in the Ayurveda and Unani systems.

Many organisms can cause several diseases and now, in this world of modern science, man can face many challenges against any disease. But in spite of the tremendous advancement of medical science and technology, diseases are the leading health problem particularly in the under privileged population in the remote and rural areas in the developing countries. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many of them based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world. They have been used as a source of medicine. The wide

spread use of herbal remedies and health care preparations, such as those described in ancient texts like Vedas and the bible, has been traced to the occurrence of natural products with medicinal properties. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Plants with possible anti-bacterial and anti-fungal activities should be tested against an appropriate microbial model. Due to the fact that the plant which is selected for investigation is very useful, as found by above mentioned reports and the fact that little information cited in the literature is available on the biological activities, there is a need to find out more about the potentiality of this plant as an antibacterial and anti-fungal agent. The present study is, therefore, designed to assess the potency of different solvents extracts of bark of soymida febrifuga on some selected microorganisms.

MATERIALS AND METHODS

Instruments used: Analytical balance, Hot air oven, Autoclave, Laminar air flow, Incubator, Micropipettes. Antibacterial and antifungal assays were carried out by adopting the agar well diffusion method [7] for the crude extracts and standard disc diffusion technique [8] for the crude extracts of selected organic solvents on Mueller-Hinton agar (MHA) for bacteria and Sabouraud Dextrose Agar (SDA) for the fungus.

Sources of the Plant Materials

Plant samples: The bark of *soymida febrifuga* was collected from the Addateegala forest, East Godavari District, A.P., India. They are chopped into pieces and are properly cleaned with water and dried. After 2-3 days of drying, the bark is pulverized into fine powder by grinding machine. Fine powder thus obtained is stored in air tight containers.

Procedure: The organic solvents were used are n-hexane, dichloromethane, methanol and ethyl acetate. All solvents used for this study were redistilled and purified in Chemistry Department V.S.M. College. The powder material is soaked in the selected organic solvents and, they are filtered by the filter paper. The filtrate was taken into the round bottom flask of the distillation unit. It is kept into water bath and heated by the heating mantle with the help of electrical power is switched on. The distillation process is started and the solvent in the flask will be boiled and the vapours are collected. After complete vapourization of the organic solvent in the flask, the remnant was taken as the crude extract of the bark in selected organic solvent. This extract was collected in air tight container. The same process is followed for all solvents to get the crude extract of the *soymida febrifuga*. Anti-bacterial activity and anti-fungal activity were investigated with the help of Microbiology Department of V.S.M. College, Ramachandrapuram. Selected organisms were collected for present study from MTCC, Chandigarh, India for the testing of crude extracts are Gram positive and Gram negative Bacterial Species of Escherichia coli, Klebsiellapneumonia, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus and Fungal species of Aspergillus fumigatus, Fusarium graminearum, Saccharomyces cerevisiae, Mucor piriformis, Microsporum canis.

Revival of the Pure Cultures: Take the lyophilized pure culture of bacteria. Prepare sterile nutrient broth and sterile nutrient agar slants. Break open the vials and add 100 µL of sterile nutrient broth and mix it. Take a loopful of the culture and streak on the solidified nutrient agar slants. They are incubated at 37^0C for 24 h.

Preparation of 24 h Pure Cultures: Prepare five conical flasks of nutrient broth of 10mL each. They are sterilized by autoclaving at 121^oC for 15 min. After autoclaving they are cooled. Take a flask inoculate a loopful of the desired slant culture and incubate the flask 37^0 C for 24 h.

Anti-Bacterial and Anti-Fungal Testing of the Crude Extracts

The steps involved are as follows: Prepare one Muller Hinton agar plate and one nutrient agar plate. To this add 0.1 ml of the inoculums, spread it. They are kept in an incubator for 10 min for the setting of the cultures on the plates. Now with a cork borer wells/cups are made. To this add 10µL of the desired organic solvent bark extract. These are placed in an incubator at 37°c for 18-24 h. After the incubation period zone of inhibition was seen. Measure the zone of inhibition.

Preparation of stock solution of root extracts of *soymida febrifuga:* Weigh 0.005g of crude root extracts of *soymida febrifuga*. Dissolve in 1000µL of different organic solvent extracts (Ethyl acetate, Methanol, n-Hexane and CH2Cl2).This gives the stock solution of *soymida febrifuga*.

Preparation of working standard solutions of *soymida febrifuga:* Take 100 µL of the stock solution. Make up to 1000 µL by adding 900 µL of desired solvent. This will give a concentration of 400 µg μL^{-1}.

Process of studying the anti-bacterial activity and anti-fungal activity of the desired concentrations of the crude root extracts: The process involves the following steps-
1. Preparation of the liquid cultures of the desired organism.
2. Preparation of lawn cultures of microorganisms.
3. Loading with the organic solvent root extract.

Preparation of the liquid cultures of the desired organism: Prepare 6 flasks of nutrient broth each containing 10 mL of the media. Sterilize by autoclaving at 121° C for 15 min. After they get cooled inoculate a loopful of culture from the nutrient agar slant. They are incubated at 37°C for 18-24 h.

Preparation of lawn cultures of microorganisms on nutrient agar & Muller Hinton agar: Prepare 150 mL of nutrient agar & Muller Hinton agar in 250 mL conical flasks. They are plugged and autoclaved at 121° C for 15 min. After sterilization they are poured into 7 sterile Petri plates. After they get solidified the desired liquid culture was swabbed in both horizontal and vertical direction. They are left for few minutes in incubator. A lawn culture of desired organism was obtained.

Loading with the organic solvent root extract: On to the solidified agar plates make wells/cups by using cork borer. To these cups add 10 µl of working standards of desired organic solvent bark extracts. They are incubated in the normal position at 37° C for 18- 24 h. After the incubation period zone of inhibition was measured.

Table: 1: Anti –bacterial activity of different crude bark extracts against Bacterial species (Zone of inhibition in mm)

Bacterial species	Ethyl acetate	Methanol	n-Hexane	CH_2Cl_2
Escherichia coli	11 mm	NZ	9mm	NZ
Klebsiella pneumonia	NZ	8mm	NZ	NZ
Pseudomonas aeruginosa	8 mm	NZ	NZ	NZ
Salmonella typhi	10 mm	NZ	NZ	NZ
Staphylococcus aureus	13 mm	15 mm	8 mm	NZ

NZ = No Zone of Inhibition

Table: 2 Anti-fungal activity of different crude bark extracts against fungal species (Zone of inhibition in mm)

Fungal species	Ethyl acetate	Methanol	n-Hexane	CH2Cl2
Aspergillus fumigatus	NZ	NZ	NZ	NZ
Fusarium graminearum	NZ	NZ	NZ	NZ
Saccharomyces cerevisiae	8mm	NZ	NZ	NZ
Mucor piriformis	9mm	NZ	NZ	NZ
Microsporum canis	NZ	NZ	NZ	NZ

NZ = No Zone of Inhibition

RESULTS AND DISCUSSION

As can be seen from table 1, Among the extracts assayed, the Ethyl Acetate bark extract of Soymida febrifuga exhibited good activity against Escherichia coli (11mm), Salmonella typhi (10mm), Staphylococcus aureus (13mm) and Pseudomonas aeruginosa (8mm) except to the Klebsiella Pneumonia (NZ).

The Methanol root extract of *Soymida febrifuga* exhibhited significantly higher activity against Staphylococcus aures (15mm) and moderately on Klebsiella Pneumonia (8 mm). Methanol bark extract of *Soymida febrifuga* shows no zone of inhibition against *Escherichia coli. Salmonella typhi*, *Psedomonas aeruginosa*. N-hexane bark extract of *Soyamida febrifuga* exhibited good activity against *Escherichia coli* (11mm) and *Staphylococcus aureus* (8mm). Klebsiella pneumonia, Pseudomonas aeruginosa and Salmonella typhi shows no activity against n-hexane bark extract of Soyamida febrifuga.

Methylene chloride bark extract of *Soyamida febrifuga* does not shows any Zone of inhibition against any bacterial species. So it has been no antibacterial activity. The results are compared with that of Kanamycin as a standard antibiotic. Of the four extracts, Ethyl acetate Extract (EER) showed more antibacterial activity against Gram positive and Gram negative bacteria (13mm, 11mm) and Methanol extract showed only against gram positive bacteria (15mm). Sensitivity of the bacteria to the other different extract is as follows

Klebisella pneumonia*:* It is moderately sensitive to methanol extract compared to Ethyl acetate,n-hexane and Methylene chloride extracts. Pseudomonas aeruginosa*:* It is moderately sensitive to the Ethyl acetate extract.Salmonella typhi *:* It is good sensitive to ethyl acetate extract. Staphylococcus aureus : It is highly sensitive to ethyl acetate, Methanol extracts and moderate to n-hexane. The present study of anti-bacterial activity also extended to diluted solutions of all the four extracts but zone of inhibition decreased and almost nil at crude 10µL and solvent 100µL quantity.

As can be seen from table 2, among all the fungal species only Saccharomyces cerevisiae and Mucor piriform were showned anti-fungal activity against Ethyl acetate extract. On an overall Consideration, Ethyl acetate extract (EER) of bark of Soyamida febrifuga showed more antibacterial activity as compared to Methanol, n-hexane and Methylene chloride extract.

APPLICATIONS

The above investigation showed that the crude extracts obtained from bark of *Soymida febrifuga* can be used enough as drug to treat disease caused by those of bacteria

CONCLUSIONS

It may, therefore be concluded from above investigation that the crude extracts obtained from bark of Soyamida febrifuga may be used enough as drug to treat disease caused by those of bacteria which showed sensitivity to the above mentioned samples. However further specific studies are needed to better evaluate the potential effectiveness of the crude extracts as antimicrobial and anti-fungal agents.

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