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RESEARCH ARTICLE

Antimicrobial Activity of Isolated Stigmasterol from Methanol Extract of Acalypha Indica

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ABSTRACT

The present study deals with the leaf extracts of Acalypha indica were tested against bacteria by disc diffusion method. Antimicrobial activity of isolated Stigmasterol from methanol extract of Acalypha indica was studied using solvent methanol against bacterial strains like Staphylococcus aureus, Bacillus subtilis, Escherichia coli and salmonella typhi. The isolated Stigmasterol from methanol extract of Acalypha indica showed the maximum zone of inhibition against E. coli and, minimum inhibition of Bacillus substilis. The isolated Stigmasterol from methanol extract of Acalypha indica showed maximum inhibition against Escherichia coli, Bacillus subtilis and Staphylococcus aureus was resistant to Stigmasterol.

Keywords: Antimicrobial activity, *Acalphaindica*, isolated Stigmasterol, streptomycin

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CONTENTS

1. Introduction

Medicinal plants constitute an effective source of both traditional and modern medicines. About 80% of rural population depends upon the herbal medicine for their primary health care. The world health organization complied more than 20,000 species of medicinal plants. Indian medicinal plants and their products are used to control the various types of diseases such as bronchitis, pneumonia, diarrhea and ulcer[1]. Medicinal plants are plants containing inherent active ingredients used to cure disease **[2]**. The use of traditional medicines and medicinal plants mostly used in developing countries for maintained of good health (UNESCO, 1996). Medicinal plants produce bioactive compounds used mainly for medicinal purposes. These compounds either act on different system of animals and man and act through interfering in the metabolisms of microbes infecting them. The microbe may be pathogenic

or symbiotic. The bioactive compounds play an important role in regulating host microbe interaction in favor of the host. The medicinal properties of plants could be based on the antioxidant, antimicrobial, antipyretic effect of the phytochemicals in them**[2]** .Acalypha indica known as kuppaimani in tamil is an annual weed. It belongs to the family Euphorbiaceous. It is an common weed in many parts of Asia. It grows in the common farmlands, gardens, roadside waste lands. Parts used are leaves, root, stalk and flowers. The major phytochemical constituents are alkaloids acalypus and aclyphine [3] .This plant is used as diuretic, antihelmintic and for respiratory problems such as bronchitis, asthma and pneumonia [4].

2. Materials and methods

Plant Material

The whole plants of Acalypha indica Linn. were collected from Cherlapally Nalgonda rural area Hyderabad road, Telangana in the month of September. Botanical identification was done by Prof Badraiah, Department of Botany, Osmania University, Hyderabad. The plant material was shed dried and powdered to coarse material and subjected to hot continuous extraction using Soxhlet apparatus method with various solvents. From all the solvent extraction, the yield of methanol extract was good hence the methanol extract was selected for further evaluation and isolation.

Isolation of compound from extract.

Methanol extract was subjected to TLC and HPTLC method to isolate the phytosterol using optimized solvent ratio is chloroform and ethanol (9.5:0.5). From laboratory studies and other analytical techniques like IR, HPLC, NMR Mass, the isolated Phytosterol was identified as Stigmasterol. Further same solvent ratio is used to isolate the Stigmasterol through column chromatography. The isolated compound was used for the antimicrobial study.

Evaluation of anti-microbial activity:

1.5 Bacterial Strains

The test organisms were obtained from the Department of Microbiology, Osmania University, Hyderabad, and Telangana, India. Two Gram-positive (Staphylococcus aureus, Bacillus substilis) and Two Gram-negative (Escherichia coli and salmonellatyphi) bacterial strains were used in the study. The organisms were sub-cultured on Agar medium, incubated at 37°C for 24 h and stored at 4°C in the refrigerator to maintain stock culture.

Disc diffusion method:

In this method, filter paper discs are used and in which the test compound containing desired concentration is placed in the agar surface. Here the Petri dishes are supposed to be incubated under certain suitable conditions. The anti-microbial agents gets diffuses into the agar surface, which thereby reduces the growth of test micro organism. Finally the diameter of the inhibited growth zone is measured and the growth media temperature is noted **[5].** The disk diffusion method is used to evaluate antimicrobial activity of the isolated stigma sterol. The isolated stigma sterol (50 mg) were re- dissolved in 2.5 ml of ethanol, sterilized through Millipore filter (0.22 µm) then loaded over sterile filter paper discs to obtain final concentration of 10 mg/disc. Agar medium was poured into sterile Petri dishes followed with 15 ml of seeded medium previously inoculated with bacterial suspension to attain bacterial medium. Sterile filter paper discs loaded with isolated stigma sterol with different concentration were placed on the top of agar plates. Filter paper discs loaded with 20µg of streptomycin was used as standard. The plates were kept in the fridge at 5 °C for 2 hrs to permit isolated stigma sterol diffusion then incubated at 37 °C for 24 h. The presence of inhibition zones were measured by Vernier calipers, recorded and considered as indication for antibacterial activity.

3. Results and discussion

The antibacterial activity of isolated Stigmasterol compound was determined by using disc diffusion methods. The results in Table 1.1 show that the isolated compound has good antibacterial activity against selected bacteria strains. In the present study, the inhibitory action of the Stigmasterol was found to increase with an increase in concentration against all bacterial strains. In vitro antibacterial activity of isolated compound from methanol extract of Acalypha indica Linn i.e. stigma sterol and standard streptomycin is given in Table – 5.7. Depicted Results showed that stigma sterol exhibited antimicrobial activity both at 50 µg/ml, 100 µg/ml, and 150 µg/ml, per disc and at 200 µg/ml per disc concentrations for all the tested bacteria which were comparable to that of standard drug streptomycin at 20 µg/ml concentration. Large zone of inhibition in disc diffusion was found out. Antibacterial activity was more in Gram negative bacteria than Gram positive bacteria. At 200 µg/ml highest activity was noted against E.coli (14.2±0.4) and lowest activity was found for Staphylococcus aureus (11.9±0.2) Highest activity was noted against E.coli (13.5±0.21) at 150 µg/ml and lowest activity was found for Bacillus subtilis **(**10.9±0.12). Highest activity was noted against E.coli (13.1±0.23) at 100 µg/ml and lowest activity was found for Staphylococcus aureus **(**9.7±0.21). Highest activity was noted against E.coli (11.2±0.33) at 50 µg/ml and lowest activity was found for Bacillus subtilis **(**9.2±0.24). With increase in the concentration of Stigmasterol, the zone of inhibition also gets increased, and the Stigmasterol (200 µg /ml), is considered as the nearest activity to standard drug. Among the all, E.coli showed more significant antimicrobial activity when compared with the standard drug.

Table: 1.1 Antimicrobial activity of isolated Stigmasterol from methanol extract of *Acalypha indica*

Treatments	Gram positive		Gram negative	
	Staphylococcus aureus	Bacillus substilis	E.coli	Salmonella typhi
Control (2%)	7.31 ±0.32	7.21 ±32	7.21 ±00	7.31 ±32
Stigmasterol (50µg/ml)	9.5 ± 0.74	9.2±0.24	11.2±0.33	7.2±0.4

Stigmasterol (100 µg /ml)	9.7±0.21	10.1±0.32	13.1±0.23	8.3±0.1
Stigmasterol (150 µg /ml)	10.2±0.31	10.9±0.12	13.5±0.21	9.2±0.3
Stigmasterol (200 µg /ml)	11.9±0.2	12.2±0.3	14.2±0.4	11.2±0.23
Standard (streptomycin 20 µg /ml)	15.2±0.3	15.3±0.29	15.6±0.21	15.1±4.3
Treatments	Gram positive		Gram negative	
	Staphylococcus aureus	Bacillus substilis	E.coli	Salmonella typhi
Control (2%)	7.31 ±0.32	7.21 ±32	7.21 ±00	7.31 ±32
Stigmasterol (50µg/ml)	9.5 ± 0.74	9.2±0.24	11.2±0.33	7.2±0.4
Stigmasterol (100 µg /ml)	9.7±0.21	10.1±0.32	13.1±0.23	8.3±0.1
Stigmasterol (150 µg /ml)	10.2±0.31	10.9±0.12	13.5±0.21	9.2±0.3
Stigmasterol (200 µg /ml)	11.9±0.2	12.2±0.3	14.2±0.4	11.2±0.23
Standard (streptomycin 20 µg /ml)	15.2±0.3	15.3±0.29	15.6±0.21	15.1±4.3

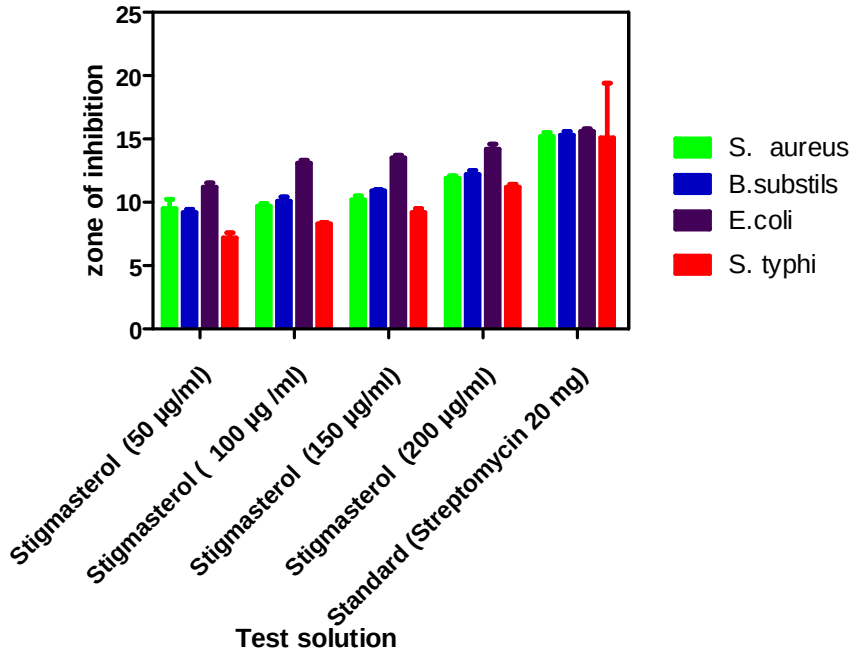

Fig:1.1 Antimicrobial activity of isolated Stigmasterol from methanol extract of *Acalypha indica* graphical representation

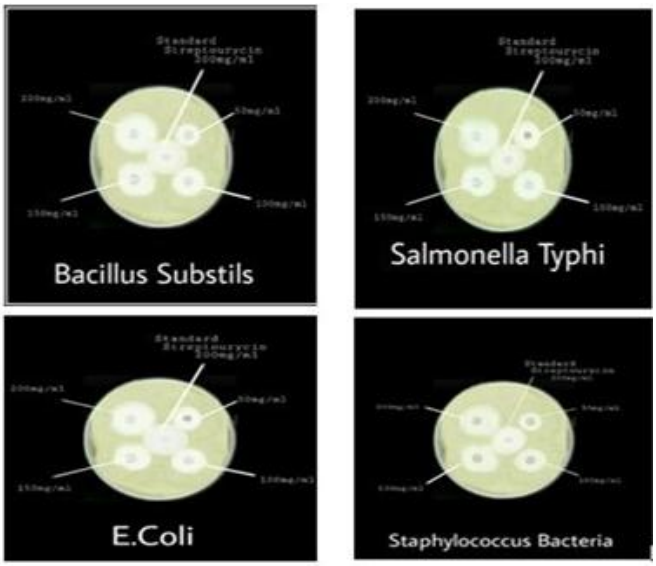

Fig.1.2

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