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ANTIMICROBIAL ACTIVITY OF TERPENOIDAL AND STEROIDAL FRACTION OF *HETEROPHRAGMA ADENOPHYLLUM* LEAF.

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ABSTRACT

The present study aimed to evaluate the *in vitro* antimicrobial activity of terpenoidal and steroidal fraction of *Heterophragma adenophyllum* leaf extract against *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus*, *Bacillus anthracis and Klebsiella pneumonial*. Study was carried out by using standard nutrient broth for microbes and streptomycine were taken as positive control. Terpenoidal and steroidal fraction of ethyl acetate extract used as test sample and DMSO as negative control. From the results of zone of inhibition it was concluded that the terpenoidal and steroidal fraction of extract were pusses antimicrobial activity while results of minimum inhibitory concentration, it was reveal that all bacterial strains were sensitive towards selected fraction.

KEYWORDS: Antimicrobial, *Heterophragma adenophyllum* terpenoid, steroid.

INTRODUCTION

The selected plant was traditional medicinal tree occurring in both tropical and sub tropical regions of the world and is important for the prevention and treatment of various diseases. In traditional medicine, the leaves are used for topical treatment of skin diseases. Fruits of *heterophragma adenophyllum were* cooked and Flowers were consumed as fresh food. The tree is extensively used in traditional medicine. As an ingredient in message oils, it is supposed to ease muscular tension sparingly cultivated as an ornamental tree. The wood is elastic and is used for making bows in Burma and also for furniture (katsagon). Folk medicinal uses of Heterophragma adenophyllum roots in Piles, constipation and also prescribed as drink in viper bite.[1-3]

α-Lapachone was previously isolated from the wood of the Bignoniaceae tree *Heterophragma adenophyllum* A new symmetric naphthoquinone dimer, dilapachone, and a novel asymmetric naphthoquinone dimer, adenophyllone were isolated from the heartwood of *Heterophragma adenophyllum*. The aim of present study was to evaluate the terpenoid and steroidal reach fraction for antimicrobial study.[4-6]

MATERIALS AND METHODS

Collection of plant material

Leaves of *Heterophragma adenophyllum* was obtained and collected from Baroda, Gujarat during April-May were voucher specimen authenticated and deposited in herbal drug technology laboratory pharmacy department, The M S University of Baroda, Gujarat, India.

Preparation of extract

Leaves was collected and washed with water to remove soil and straw from base. The leaves were shade dried and coarsely powered for further process. The powdered leaves of *Heterophragma adenophyllum* was extracted with ethyl acetate by using hot percolation method.[7] The extract were oven dried at low temperature and treated with aqueous KOH solution. Then separate organic layer and treated with aqueous hydrochloric acid, this acid-base reaction were repeated twice and collected organic layer. Finally organic layer washed with water and collected. Organic layer were concentrate to 50 ml and centrifuge at 6000 rps, then supernatant fluid were collected and evaporate to dryness. Dry residues suspended to DMSO for further use.

Microorganism used

The gram positive organism *Staphylococcus aureus*, *Bacillus anthracis*, *Klebsiella pneumonia* and gram negative organism *Escherichia coli* and *Salmonella enterica* bacteria were used for antimicrobial study.[8] The all microbes were maintained in sterile conditions and grown on nutrient broth.

Preparation of slandered bacterial suspension.

Antimicrobial activity of terpenoidal and steroidal fraction of *Heterophragma adenophyllum* was carried out by determining zone of inhibition through agar well diffusion method and calculating minimum inhibitory concentration through micro dilution assay method. After growth, some colonies of microbes were selected and transferred aseptically in to the tubes and centrifuge fully

after adding sterile saline water.[9] The bacterial suspension thus obtain were compared with the 1% McFerland standard. McFerland standard was checked by using a spectrophotometer with a 1-cm light path.[10] The absorbance at the wave length 600 nm was found to be 0.129, which is near to standard 0.123.

Determination of zone of inhibition

Five sets of six sterile agar nutrient plates were taken for the study for zone of inhibition of the five microbes. Agar plates were incubated with respective test organisms. Three holes of 6 mm diameter in the media of each plate were bored. One hole was filled with streptomycin solution of 50 µg/ml concentration as the positive control, another hole with 500 µg/ml concentration of terpenoidal and steroidal fraction solution as a test while, third hole was filled with DMSO as kept for negative control. Plates were then incubated at 37^{o}C for 24 hrs. After incubation plates were examine for the presence of zone of inhibition.[11,12]

Determination of minimum inhibitory concentration by micro dilution assay method

Six dilution of the fraction ranging from 500 – 3.9 µg/ml were prepare using two fold serial dilution method. Standardized inoculation of microorganisms of 1% McFerland standard turbidity prepared 1:1000 (10^5 CFU/ml) by adding sterile saline. Diluted sterile bacterial suspension and terpenoidal and steroidal fraction of *Heterophargma adenophyllum* were added in to the wells of micro titer plates. Streptomycin[13] (50 µg/ml) was used as positive control and DMSO used as negative control while terpenoidal and steroidal fraction used as test sample and incubated at 37^{o}C for 24 hrs. Microbial growth was determined at absorbance at 600 nm using RT-2100 micro plate reader. The MIC values were taken as the lowest concentration of the fraction in the wells of the microtiter plates that shows no turbidity of the wells in the plates.

RESULT AND DISCUSION

Results of antimicrobial activity of terpenoidal and steroidal fraction of *Heterophragma adenophyllum* leaves extract by agar-well diffusion method is shown in table 1. From the results of zone of inhibition it was reveal that the terpenoidal and steroidal fraction possesses an efficient and strong antimicrobial activity against both the gram positive and gram negative bacteria. Results of the antimicrobial activity of terpenoidal and steroidal fraction by micro dilution method are shown in table 2. From the results of the minimum inhibitory concentration (table 2), it was shows that the almost all tested microorganism were found sensitive towards the terpenoidal and steroidal fraction of *Heterophragma adenophyllum* leaves extract. Phytochemical and preparative thin layer chromatography shows that various compounds of terpenoidal and steroidal nature were present in this fraction. Further studies were required for separation and isolation of active phytochemicals from terpenoidal and steroidal fraction of the leaves.

Table 1. Antimicrobial activity of terpenoidal and steroidal fraction (TSF) of *Heterophragma adenophyllum* leaf by agar well diffusion method

	Concentration	Zone of inhibition (mm)				
		E. coli,	***S. enterica,***	***S. aureus,***	***B. anthracis***	***K. pneumonial***
Positive control	50 µg/ml	31 ± 0.37	29 ± 0.31	27 ± 0.19	28 ± 0.43	25 ± 0.09
Test TSF	500 µg/ml	19 ± 0.11	15 ± 0.42	16 ± 0.26	15 ± 0.12	12 ± 0.43
Negative control		NA	NA	NA	NA	NA

NA= No activity, all values are mean ± standard deviation, N=3 (experiment in triplicate).

Table 2. Antimicrobial activity of terpenoidal and steroidal fraction (TSF) of *Heterophragma adenophyllum* leaf by micro dilution assay method

	Minimum inhibitory concentration (µg/ml)				
	E. coli,	***S. enterica,***	***S. aureus,***	***B. anthracis***	***K. pneumonial***
Positive control	0.243	0.324	0.159	0.284	0.183
Test TSF	142	139	133	211	65.5
Negative control	NA	NA	NA	NA	NA

NA= No activity.

CONCLUSION

The separated terpenoidal and steroidal fraction from ethyl acetate extract of the leaves of *Heterophragma adenophyllum* demonstrated broad spectrum antimicrobial activity against both gram positive and gram negative bacteria. This activity of fraction may be possible due to bioactive phytochemical are presence in the fraction. Bioactive compound from the fraction can be identified and isolated for further use in development of antimicrobial formulation for the treatment of various infections. Thus the present study significantly proves that terpenoidal and steroidal reach fraction can be beneficial against antimicrobial agents.

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