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IN VITRO EVALUATION OF ANTIMICROBIAL ACTIVITY OF PETROLEUM ETHER FRACTION FROM *HETEROPHRAGMA ADENOPHYLLUM* LEAVES EXTRACT

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ABSTRACT

The present study aimed to evaluate the *in vitro* antimicrobial activity of petroleum ether fraction from *Heterophragma adenophyllum* leaves extract against *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus*, *Bacillus anthracis and Klebsiella pneumonia* broad spectrum microorganism. Study was carried out by using standard nutrient broth for microbes and gentamicine were taken as positive control. Petroleum ether fraction used as test sample and DMSO as negative control. From the results of zone of inhibition it was concluded that the petroleum ether fraction were pusses *in vitro* antimicrobial activity while results of minimum inhibitory concentration, it was reveal that all bacterial strains were sensitive towards selected fraction for the study.

KEYWORDS: Antimicrobial, Heterophragma Adenophyllum, Petroleum Ether Fraction.

INTRODUCTION

The selected plant for the study was traditional medicinal tree occurring in both tropical and sub tropical regions of the world. In India it is found in forest of Maharashtra, Gujarat, Rajasthan and Assam. *Heterophragma adenophyllum* areal part 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 petroleum ether fraction of the leaves extract 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 (authen.06/2012/botany) and deposited in pharmacognosy laboratory of Maliba Pharmacy College, Uka Tarsadia University, Bardoli, 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 petroleum ether by using hot percolation method.[7] The extract were oven dried at low temperature and treated with 05% aqueous KOH solution. Then separate organic layer and treated with 10% 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 *in vitro* antimicrobial study.[8] The all microbes were maintained in sterile conditions and grown on nutrient broth.[9]

Preparation of slandered bacterial suspension.

Antimicrobial activity of petroleum ether 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.[10]

After growth, some colonies of microbes were selected and transferred aseptically in to the tubes and centrifuge fully after adding sterile saline water.[11] 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.[12] 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 gentamicin solution of 50 µg/ml concentration as the positive control, another hole with 500 µg/ml concentration of petroleum ether 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.[13,14]

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 petroleum ether fraction of *Heterophargma adenophyllum* were added in to the wells of micro titer plates. Gentamicin[15] (50 µg/ml) was used as positive control and DMSO used as negative control while petroleum ether 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

Table. 1. Antimicrobial activity of petroleum ether fraction of *Heterophragma adenophyllum* leaves extract 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 Control	500 µg/ml	22 ± 0.14	18 ± 0.32	19 ± 0.28	18 ± 0.24	16 ± 0.23
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 petroleum ether fraction of *Heterophragma adenophyllum* leaves extract 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 control	139	136	129	215	86.5
Negative control	NA	NA	NA	NA	NA

NA= No activity

Results of antimicrobial activity of petroleum ether 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 petroleum ether fraction possesses an efficient and strong antimicrobial activity against both the gram positive and gram negative bacteria. Results of the antimicrobial activity of petroleum ether 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 broad spectrum tested microorganism were found sensitive towards the petroleum ether 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 petroleum ether fraction of the leaves.

CONCLUSION

The separated petroleum ether fraction of *Heterophragma adenophyllum* leaves extract 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 the isolated fraction from the petroleum ether extract of the *Heterophragma adenophyllum* can be beneficial against antimicrobial agents.

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