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IN VITRO ANTIMICROBIAL SCREENING OF MULTIDRUG RESISTANT BACTERIA BY ISOLATED FRACTION FROM *HETEROPHRAGMA ADENOPHYLLUM* LEAVES

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ABSTRACT

The present study aimed to screen the *in vitro* antimicrobial activity of ethyl acetate fraction isolated from crude chloroform extract of the leaves of *Heterophragma adenophyllum* against Multidrug-resistant pathogenic bacteria such as *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus*, *Bacillus anthracis and Klebsiella pneumonia* broad spectrum microorganism. The study was carried out by using standard nutrient broth for microbes and streptomycin was taken as a positive control. Ethyl acetate fraction used as a test sample and DMSO as a negative control. From the results of the zone of inhibition, it was concluded that the ethyl acetate fraction from crude chloroform extract was pusses *in vitro* antimicrobial activity while results of minimum inhibitory concentration, Plant-derived, and other safe natural antimicrobial compounds have the potential to control the prevalence of both susceptible and resistant pathogens in various environments it was revealed that all bacterial strains were sensitive towards selected fraction from the plant for the study.

KEYWORDS: Antimicrobial, multidrug, *Heterophragma adenophyllum*, ethyl acetate fraction.

INTRODUCTION

In India *Heterophragma adenophyllum* tree found in the forest of Maharashtra, Gujarat, Rajasthan, and Assam. The areal part of the plant is important for the prevention and treatment of various diseases as per the traditional claims. The *Heterophragma adenophyllum* plant selected for the study was a traditional medicinal tree occurring in both tropical and subtropical regions of the world. 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 in Thailand and nearby states. The tree is extensively used in traditional medicine, as an ingredient in massage 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). The roots of the plant is used in Folk medicine as in Piles, constipation and also prescribed as a 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 was isolated from the heartwood of *Heterophragma adenophyllum.* The aim of the present study was to evaluate the ethyl acetate fraction from the crud chloroform extract of the leaves 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, the voucher specimen was authenticated (authen.06/2012/botany) and deposited in pharmacognosy laboratory of Maliba Pharmacy College, Uka Tarsadia University, Bardoli, Gujarat, India.

Preparation of extract

Leaves were collected and washed with water to remove soil and straw from the base. The leaves were shade dried and coarsely powered for further process. The powdered leaves of *Heterophragma adenophyllum* were extracted with chloroform by using hot percolation method.[7] The extract was oven dried at low temperature and fractionated with petroleum ether, ethyl acetate, and methanol. The ethyl acetate fraction was separated and evaporated to dryness. Dried ethyl acetate fraction treated with 05% aqueous KOH solution. Then separate the organic layer and treated with 10% aqueous hydrochloric acid, this acid-base reaction was repeated twice and collected organic layer. Finally, the organic layer washed with water and collected. The organic layer was concentrated to 50 ml and centrifuge at 6000 rpm, the supernatant fluid was collected and evaporated 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] All microbes were maintained in sterile conditions and grown on nutrient broth.[9]

Preparation of slandered bacterial suspension.

Antimicrobial activity of ethyl acetate fraction from crude chloroform extract of *Heterophragma adenophyllum* was carried out by determining the zone of inhibition through agar well diffusion method and calculating minimum inhibitory concentration through microdilution assay method.[10] After growth, some colonies of microbes were selected and transferred aseptically into the tubes and centrifuge fully after adding sterile saline water.[11] The bacterial suspension thus obtained 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 wavelength 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 the 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 ethyl acetate fraction solution from chloroform extract as a test while the 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 examined for the presence of zone of inhibition.[13,14]

Determination of minimum inhibitory concentration by micro dilution assay method

Six dilutions of the fraction ranging from 500 – 3.9 µg/ml were prepared 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 ethyl acetate fraction from crude chloroform extract of *Heterophargma adenophyllum* were added into the wells of micro titer plates. Streptomycin[15] (50 µg/ml) was used as positive control and DMSO used as negative control while ethyl acetate fraction used as a test sample and incubated at 37^{o}C for 24 hrs. Microbial growth was determined at an 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 micro titer plates that shows no turbidity of the wells in the plates.

RESULT AND DISCUSION

Results of antimicrobial activity of ethyl acetate fraction from crude chloroform extract of *Heterophragma adenophyllum* leaves by the agar-well diffusion method is shown in table 1. From the results of the zone of inhibition, it was revealed that the ethyl acetate fraction possesses an efficient and strong antimicrobial activity against both the gram-positive and gram-negative bacteria. Results of the antimicrobial activity of ethyl acetate fraction by micro dilution method are shown in table 2. From the results of the minimum inhibitory concentration (table 2), it was shown that the almost all broad spectrum tested microorganism was found sensitive towards the ethyl acetate fraction from crude chloroform extract of *Heterophragma adenophyllum* leaves. 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 ethyl acetate fraction of the leaves.

Table 1: Antimicrobial activity of ethyl acetate 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	42 ± 0.34	29 ± 0.33	36 ± 0.23	38 ± 0.33	33 ± 0.39
Test Control	500 µg/ml	32 ± 0.34	28 ± 0.37	29 ± 0.37	30 ± 0.34	27 ± 0.13
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 ethyl acetate 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	0.345	0.304	0.221	0.271	0.263
Test control	156	157	149	234	107.4
Negative control	NA	NA	NA	NA	NA

NA= No activity

CONCLUSION

The separated ethyl acetate fraction from the crude extract of chloroform of *Heterophragma adenophyllum* leaves demonstrated broad-spectrum antimicrobial activity against both gram positive and gram negative bacteria. This activity of fraction may be possible due to bioactive phytochemicals are presence in the fraction. Bioactive compound from the fraction can be identified and isolated for further use in the development of an antimicrobial formulation for the treatment of various infections. Plant-derived natural antimicrobial compounds have the potential to control the prevalence of both susceptible and resistant pathogens in various environments. Thus the present study significantly proves that the isolated fraction from the ethyl acetate extract of the *Heterophragma adenophyllum* can be beneficial against antimicrobial agents.

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