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IN VITRO EVALUATION OF ANTIMICROBIAL ACTIVITY OF K*nema attenuata* STEM BARK EXTRACT

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ABSTRACT

Medicinal plants have very high potential as antimicrobial drugs for treating various human diseases. The purpose of present work is to study the antimicrobial activity present in ethanolic extract obtained from stem bark of *Knema attenuata.* Microorganisms used for test were *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans.* The antibacterial and antifungal activities of extract were studied using agar well diffusion and disc diffusion techniques. The zone of inhibition of growth was measured and recorded. The results obtained indicated the presence of mild antibacterial but moderate antifungal activity in the stem bark extract. Therefore the stem bark of *K.attenuata* can act as a natural antifungal agent.

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INTRODUCTION

Infectious diseases caused by microorganisms like bacteria; fungi etc are becoming a major health problem and also causing burden to individual and the nation. A pathogen is a micro-organism which upon invasion and multiplication in an individual or in a population causes infection; and when this infection causes damage to individual's vital systems disease will occur. A wide range of antimicrobials are available in market for the positive control of these microorganisms. Antimicrobials have reduced the morbidity and mortality due to microbial infections, by attacking pathogenic agents either by disrupting bacterial cell wall or inhibiting the enzymes or inhibiting their protein synthesis. Nowadays the drugs that were initially effective against microbes lose their effectiveness overtime when the microbes become resistant to the drug. Thus increasing prevalence of certain resistant pathogenic microbes remind about the need for developing new antimicrobial agents in near future [1].

Knema attenuata or *Myristica attenuata* (f. Myristicaceae) commonly known as wild nutmeg, is an endemic tree species found in southern parts of India [2]. It's one of the ingredients of ayurvedic 'Ashwagandhadi nei' used for treating spleen disorders, breathing disorders and tastelessness [3]. Anacardic acid is a common constituent found in plants under genus Knema which have antibacterial, anticancer and anti inflammatory properties [4]. A lignan attenuol have been isolated from the stem bark of *K.attenuata*; closely related to lignans isolated from plants of Myristicaceae family [5]. Chloroform extract of aril and hexane extract of seed exhibited highest antimicrobial activity against Staphylococcus aureus with a zone of inhibition of 20.0±0.66mm and moderate antifungal activity against Candida albicans(6.0±0.5mm) [6]. But very less studies have done in the stem bark of K.attenuata till now. Therefore the present study was designed to evaluate the antimicrobial activity of ethanolic stem bark extract of K.attenuata.

MATERIALS AND METHODS

Plant material

The plant for proposed study, *K.attenuata* was collected from Kerala forest Research Institute, Thrissur in the month of January 2015.The taxonomic authentication was done by Dr. V.B Sreekumar, Scientist (Department of Botany), Kerala forest Research institute, Thrissur.

Fresh stem barks were collected from the plant which is spread in trays and air dried for three weeks. Dried stem bark was coarsely powdered and stored for extraction.

Preparation of crude ethanolic stem bark extract (ESBE)

Plant extraction was carried out by soxhelation in which stem bark powder was packed well in thimble and loaded in a soxhlet apparatus. After defatting stem bark powder with petroleum ether (60-80° C), extraction was carried out with 90% ethanol. 80% of the solvent was recovered by distillation and remaining solvent was removed by heating on a water bath. The dried extract was stored free from moisture in a dessicator over activated silica gel for further studies [7]. The extract was subjected to qualitative chemical tests for the detection of various phytoconstituents like carbohydrates, glycosides, flavonoids, phenolic compounds, steroid, alkaloids etc.

Antimicrobial screening methods

Test Microorganisms

The bacterial and fungal species used for the test:-

- *Staphylococcus aureus* (*S. aureus*)
- *Escherichia coli* (*E. coli*)
- *Candida albicans (C.albicans)*

Preparation of Plant Extracts:

Ethanolic stem bark extract of *Knema attenuata* was weighed accurately and dissolved in DMSO and made up the volume with water such that the solution contains 1000 µg/ml [8-10].

Antibacterial activity

Preparation of stock culture

Constituents	(gm/L)
Peptone	5.0
Sodium chloride	5.0
Beef extract	1.5
Yeast extract	1.5
Agar	15
Distilled water	1000ml
pH was adjusted to 7.4 ± 0.2	

A loopful of the organism was taken from the cultures which were maintained on nutrient broth solution and aseptically transferred to 100 ml of sterile nutrient broth which was shaken thoroughly and incubated at 37°C for 24 hours.

Preparation of culture media

The nutrient agar medium used for the growth of bacteria was prepared by dissolving 14 g of nutrient agar in 100 ml of distilled water and was sterilized in an autoclave at 120° C for 1 hour.

Composition of Nutrient Agar Medium.

Constituents	(gm/L)
Peptone	5.0
Sodium chloride	5.0
Beef extract	1.5
Yeast extract	1.5
Agar	15
Distilled water	1000ml
pH was adjusted to 7.4 ± 0.2	

Screening for antibacterial activity

Screening of antibacterial activity was done by cup plate method. The nutrient agar culture medium was inoculated with organisms from stock culture separately by pour plate method. The medium was shaken and transferred to labelled sterile petridishes and allowed to set. Using a sterile cork borer of 5mm diameter, 5 wells were made in each petridish.50 µl of different concentrations of test solution, standard and control were applied in respective cups of plate. Solvent used for the preparation of the sample solution was used as the control. Gentamicin 100 µg was employed as standard. The plates were then left undisturbed to allow complete diffusion of the samples. These were then incubated in inverted position for 18-24 h at 37° C. After the incubation period zone of inhibition of growth was measured and recorded [11].

Antifungal activity

Preparation of stock culture

The organisms were maintained on Sabouraud's dextrose agar. One loopful of the culture in slants which were maintained below 4°C was taken and inoculated in the broth and incubated at 37°C for 24 hours and were observed for the growth of the organism with the naked eye for their turbid nature and compared with sterile broth. The presence of turbidity indicates growth and suitability of the culture for further work. From the culture which was maintained on Sabouraud's dextrose agar slants, one loopful of the respective organisms were taken and aseptically transferred to 100ml of sterile Sabouraud's dextrose broth which was shaken thoroughly and incubated at 37°C for 24hours. This is the stock culture or sub-culture.

Preparation of culture media

The sabouraud's dextrose agar media was prepared by dissolving 6.5g of sabouraud's dextrose in 100 ml distilled water and sterilized by autoclaving at 120°C for 1 hour.

Composition of Sabouraud's dextrose agar media.

Constituents	(gm/L)
Mycological peptone	10
Dextrose	40
Agar	15
Distilled water	up to 1000 ml

Screening for antifungal activity

Disc diffusion method was employed for the screening of antifungal activity. The sabouraud's dextrose agar media was transferred to petridishes; allowed to set and inoculated with organism by swabbing uniformly over the media using a sterile cotton swab.50µl of standard, control and different concentrations of test solution were applied to sterile discs and aseptically transferred to the plate. Solvent used for the preparation of sample solution was used as control. Clotrimazole 100µg was employed as standard. The plates were then left undisturbed to allow complete diffusion of samples. These were then incubated in inverted position for 48 hours at 37°C. The plates were then examined for any zone of inhibition of growth.

RESULTS

Table 1. Percentage yield obtained from *K.attenuata.*

Sl. No.	Name	Percentage yield
1.	ESBE	17.8%w/w

Table No: 2. Preliminary phytochemical studies on *K.attenuata.*

Sl. No.	Phytoconstituents	ESBE
1.	Carbohydrate	+
2.	Alkaloids	+
3.	Glycosides	+
4.	Flavanoids	+
5.	Saponins	+
6.	Phenolics	+
7.	Tannins	+
8.	Steroids	+
9.	Triterpenoids	_

Table no.3. Zone of inhibition of antimicrobial activity.

Group	Drug	Zone of inhibition (mm)		
		S. aureus	E.coli	C.albicans
Control	-	-	-	-
Standard	Gentamicin (50 µg/ml)	30	28	-
	Clotrimazole (0.1%)	-	-	18
Test	ESBE(100 µg/ml)	6	0	10

Fig.1. Antifungal activity of ESBE.

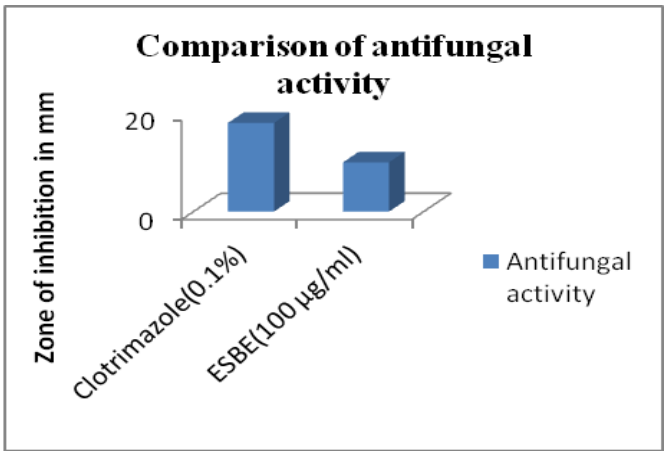

Fig.2. Comparison of antifungal activity.

DISCUSSION

Upon soxhelation, ethanolic extract was obtained with a yield of 17.8% w/w. Preliminary phytochemical screening of ethanolic stem bark extract showed the presence of various bioactive components like alkaloids, carbohydrates, cardiac glycosides, flavanoids, steroids, saponins and poly phenolic compounds.

Ethanolic stem bark extract of *Knema attenuata* showed only mild antimicrobial activity. Two bacterial species were used for screening of antibacterial activity, of which only *S.aureus* showed mild sensitivity towards the ethanolic extract. *C.albicans* was the fungal strain used for screening antifungal activity, for which the ethanolic extract showed better results. Other species of Knema like *K.angustifolia, K.furfuraceae* are also known to have antibacterial activity [12-13].

CONCLUSION

The present study was conducted to study the in vitro antimicrobial activity of ethanolic stem bark extract of plant *K.attenuata* which is used in folk medicine for the treatment of many disorders. Previous studies done on seeds and arils of plant supported the antimicrobial activity of plant. Ethanolic extract of stem bark of *K.attenuata* showed moderate antifungal activity and only a mild antibacterial activity. This study confirms the use of stem bark of plant as an antifungal agent.

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