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Antibacterial and Antifungal Activity of Heartwood of *Acacia catechu* of Nepal

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Abstract

Antibacterial activity of different extracts of heartwood of Acacia catechu were tested against 11 species of bacteria and antifungal activity were tested against four species of fungi. Among bacteria species highest zone of inhibition (ZOI) was measured against Pseudomonas species by diethyl ether extract, likewise among fungal organism highest zone of inhibition (ZOI) was measured against Fusarium oxysporim by ethyl acetate extract. No extracts showed activity against Salmonella typhi, Salmonella paratyphi, Escherichia coli and Proteus mirabilis. The minimum bactericidal concentration (MBC) of ethyl acetate extract was evaluated against Bacillus subtilis, Klebsiella pneumonia, Staphylococus aureus and Shigella species.

Keywords: *Heartwood of Acacia catechu, Zone of Inhibition, Bio-fungicides, Antibacterial, Antifungal.*

Introduction

Acacia catechu also known as cutch tree belongs to family Fabacea (Leguminosae)[1]. It contains many biologically active constituents like catechin, epicatechin, kaempferol, dihydrokaempferol, quercetin, dihydroquercetin[2], catechutannic acid, tannins[3] etc. Catechin presents in *Acacia catechu* possess significant antioxidant and antimicrobial effect[4]. It also possesses cyclooxygenase-2 and 5-lipoxygenase enzyme inhibitory effect which are ultimately responsible for rheumatoid arthritis, osteoarthritis, alzeimer's disease and certain type of cancer[5]. Epicatechin improves the blood flow which has potential for cardiac health[6]. Low concentrations of condensed tannins suppress the growth of MCF-7 breast cancer cells, and effect was related to their activity of fatty acid synthase (FAS) inhibition[7].

In folk medicine, heartwood of *Acacia catechu* is used as anti-helmintic, antiseptic, anti-dysentric, anti-inflammatory, antipyretic, haemostatic, haematinic[8]. It is also used as astringent, depurative, appetizer, tonic and considered to treat catarrh, cough, leprosy, pruritus, anorexia, pharyngodynia ect[8,9].

In this study methanol extract was obtained by cold percolation. Thus obtained extract was further extracted with hexane, chloroform, diethyl ether and ethyl acetate in order of increasing polarity to give respective extract. They were finally tested for their antimicrobial activities. For

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the efficiency of antibacterial activity minimum bactericidal concentration (MBC) of ethyl acetate extract was also determined against some bacteria species.

Experimental Method

Collection of Plant Material

The plant material was collected from Chitwan National Park, Nepal and was identified as *Acacia catechu* by using different taxonomic literatures [9,10].

Preparation of Extracts

Heartwood of *Acacia catechu* was chopped in to small pieces and dried in shade for one week. They were then grinded in grinder to obtain in powdered form. 500 gm of powder of heartwood of *Acacia catechu* was cold percolated using 1.5 liter of methanol. Methanol extract was concentrated in rotary evaporator under reduced pressure. Thus obtained methanol extract was successively extracted with hexane, chloroform, diethyl ether and ethyl acetate to give respective extract. Remaining marc was taken as methanol extract.

Preparation of Working Solution

5% of working solution was prepared by transferring 5 mg of each extract to sterile vial aseptically containing 1 ml DMSO solvents, which were then capped sealed and stored in refrigerator until use.

Microorganism Used

Study includes 11 different species of bacteria and 4 different species of fungi. Among bacteria taken on study, two were gram positive and remaining were gram negative as given below:

Gram positive bacteria: *Staphylococcus aureus and Bacillus subtilis.*
Gram negative bacteria: *Salmonella paratyphi, Escherichia coli, Pseudomonas sp., Enterobacter sp., Salmonella typhi, Shigella sp., Acenetobacter sp., Proteus mirabilis, Klebsiella pneumonia.*

This study also included 4 species of fungal organism namely *Fusarium oxysporium, Fusarium moniliformi,Fusarium proliferatum* and *Exherlium turticum.*

Antibacterial Activity

Inhibition of bacterial growth was tested by agar well diffused method as given by Dingel et. al.[11]. Already prepared Sterile Muller-Hinton Agar (MHA) plates were dried to remove excess of moisture from the surface of the media. Sterile cotton swab was dipped into the prepared inoculums and the excess of inoculums were removed by pressing and rotating against the upper inside side wall of the tube above the liquid level and then swabbed carefully all over the plates. The plate was rotated through an angle of 60^o after each swabbing. Finally the swab was passed round the edges of the agar surface. The inoculated plates were left to dry for few minutes at room temperature with the lid closed[11].

The wells were made in the incubated media plates with the help of sterile cork borer (6 mm) and labeled properly. Then 50 µl of the working solution of the plant extracts were loaded

into the respective wells with the help of micropipette. The solvent itself was tested for its activity as a control at the same time in the separate well. The plates were then left for half an hour with the lid closed so that extracts diffused to the media. The plates were incubated overnight at 37^oC[12]. The plates were then observed for zone of inhibition (ZOI) produced by the anti-bacterial activity of different extracts of heartwood of *Acacia catechu*. At the same time ZOI of different organism by different fractions were measured with the help of the ruler for the estimation of potency of anti-bacterial substance. MBC of ethyl acetate extract against *Bacillus subtilis, Klebsiella pneumonia, Staphylococus aureus, Shigella sps.* was determined by two fold dilution method.

Antifungal Activity

Inhibition of fungal growth was also tested by agar well diffused method. Sterile potato dextrose agar (PDA) plates were prepared. Before using the plates, they were dried in hot air oven at 40^oC for 5 minutes to remove excess of moisture from the surface of the media. Sterile cotton swab was dipped into the prepared inoculums and the excess of inoculums were removed by pressing and rotating against the upper inside side wall of the tube above the liquid level and then swabbed carefully all over the plates. The plate was rotated through an angle of 60^o after each swabbing. Finally the swab was passed round the edges of the agar surface. The inoculated plates were left to dry for few minutes at room temperature with the lid closed[12].

The wells were made, working solutions of the plant extracts were loaded and the solvent itself was tested for its activity as a control at the same time in the separate well as in antibacterial activity. The plates were then left for half an hour with the lid closed so that extracts diffused to the media. The plates were incubated for seven days at 27^oC[13]. The plates were then observed for zone of inhibition (ZOI) produced by the anti-fungal activity of different extracts of heartwood of *Acacia catechu*. At the same time ZOI of different organism by different extracts were measure with the help of the ruler for the estimation of potency of anti-fungal substance.

Result and Discussion

The results of the antibacterial activity are presented in Table 1. No zone of inhibition was found in control. No extract showed activity against *Salmonella typhi, Salmonell paratyphi, Escherichia coli and Proteus mirabilis*. Highest zone of inhibition was measured against *Pseudomonas species* by diethyl ether extract. From the Table 1 we can observe hexane and chloroform showed moderate activity while methanol, diethyl ether and ethyl acetate extract showed good inhibition. From these results we can say medium polar and polar constituents present in heartwood of *Acacia catechu* have good antibacterial activity than non-polar compounds.

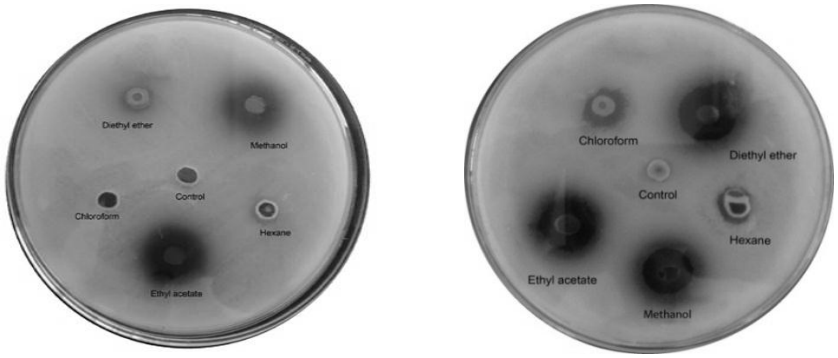
Table 2 shows minimum bactericidal concentration (MBC) of the ethyl acetate extract. Here ethyl acetate extract has good inhibitory effect against *Bacillus subtilis* and *Shigella sp.* with MBC value 50 mg/ml and then against *Klebsiella pneumonia* and *Staphylococus aureus* with MBC value 100 mg/ml.

Table 1: *Results of antibacterial activity of 5% solution of different extracts of heartwood of Acacia catechu*

Test Organisms	Zone of Inhibition (mm)				
	Hexane extract	Chloroform extract	Diethyl ether extract	Ethyl acetate extract	Methanol extract
Staphylococus aureus	-	8	10	12	11
Bacillus subtilis	7	9	13	14	12
Salmonella paratyphi	-	-	-	-	-
Escherichia coli	-	-	-	-	-
Pseudomonas sp.	-	-	15	10	9
Enterobacter sp.	9	-	8	8	8
Salmonella typhi	-	-	-	-	-
Shigella sp.	9	8	13	14	12
Acenetobacter sp.	-	7	8	12	-
Proteus mirabilis	-	-	-	-	-
Klebsiella pneumoniae	-	-	8	13	12

Table 2: *Minimum bactericidal concentration (MBC) value of Ethyl acetate extract*

Fraction	Organisms	MBC (mg/ml)
Ethyl acetate	*Bacillus subtilis*	50
	Klebsiella pneumonia	100
	Staphylococus aureus	100
	Shigella sps	50



A **B**

Figure 1: *zone of Inhibition by different extracts of heartwood of Acacia catechu against A-Klebsiella pneumoniae B- Bacillus subtilis.*

Table 3 shows the result of antifungal activity. Highest zone of inhibition was shown by ethyl acetate fraction against *Fusarium oxysporium.* Here hexane fraction showed activity only against Fusarium moniliformi. Hexane showed poor activity, chloroform and methanol showed moderate activity and diethyl ether and ethyl acetate showed good activity against fungal organism.

Table 3: *Results of antifungal activity of 5% solution of different extracts of heartwood of Acacia catechu*

Test Organism	Zone of Inhibition (mm)				
	Hexane extract	Chloroform extract	Diethyl ether extract	Ethyl acetate extract	Methanol extract
Fusarium oxysporium	-	9	10	17	10
Fusarium moniliformi	8	9	11	9	-
Fusarium proliferatum	-	-	10	9	7
Exherlium turticum	-	10	11	14	8

Zone of inhibition of ethyl acetate extract, which showed good inhibitory effect, against disease causing bacteria is depicted in Table 1. Highest zone of inhibition (14 mm) was recorded against *Shigella species* and *Bacillus subtilis* followed by *Klebsiella pneumoniae* (13 mm), *Acenetobacter species* (12 mm) indicating good antibacterial potency of the extract against these bacteria. Hexane and chloroform extract showed very small zone of inhibition against bacteria species reflecting poor antibacterial potency. Similarly different zone of inhibition

C **D**

Figure 2: *zone of Inhibition by different extracts of heartwood of Acacia catechu against C- Fusarium oxysporium D- Fusarium moniliformi.*

against fungal organism is depicted in Table 3. Here ethyl acetate extract showed very good zone of inhibition in comparison to other extracts. Highest zone of inhibition (17 mm) was measured against *Fusarium oxysporium* by ethyl acetate extract. *Fusarium oxysporium* is a

causative agent of Panama disease, disease of banana, and this fungi is resistant to commercially available fungicides. Hexane extract showed activity against only Fusarium moniliformi and no activity against other fungal organism. These reflect the good antifungal potency of ethyl acetate extract and negligible antifungal potency of hexane extract.

Conclusion

Result showed that different extracts of *Acacia catechu* have good antibacterial and antifungal properties. We observed very good zone of inhibition against fungal organism by certain extracts, so these extracts can also be used as bio-fungicides. Ethyl acetate extract showed a significant inhibitory effect against *Fusarium oxysporium*, causative agent of panama disease.

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