

**A STUDY OF THE BACTERICIDAL PROPERTY OF AQUEOUS EXTRACT OF MEDICINAL PLANTS-ACACIA CATECHU AND FISCUS HISPIDA USED IN THE TRADITIONAL SYSTEM OF MEDICINE IN MANIPUR**H. Lokhendro Singh<sup>1</sup>, S. Lokendrajit Singh<sup>2</sup>, S. Rita Devi<sup>3</sup>, Ng Brajachand Singh<sup>4</sup>, L. Supriya<sup>5</sup>**HOW TO CITE THIS ARTICLE:**

H. Lokhendro Singh, S. Lokendrajit Singh, S. Rita Devi, Ng Brajachand Singh, L. Supriya. "A Study of the Bactericidal Property of Aqueous Extract of Medicinal Plants-Acacia Catechu and Ficus Hippida used in the Traditional System of Medicine in Manipur". Journal of Evolution of Medical and Dental Sciences 2014; Vol. 3, Issue 31, July 31; Page: 8601-8607, DOI: 10.14260/jemds/2014/3093

**ABSTRACT: OBJECTIVE:** To find out the bactericidal effect of extract of the Acacia catechu and Ficus hispida which are commonly used by the local quacks for the treatment of diarrhea and dysentery in the villages of the state of Manipur where the medical facilities are not available. **METHODS:** The plant barks are collected from different parts of Manipur during the period of March 2006 to February 2007, dried at room temperature; make powder in the grinder and soxhlated at low temperature to get the yield (extract). In case of Acacia catechu dried power form is available in the market. The powder is soxhlated to get the yield. These extracts are tested for the bactericidal effect in serial dilutions against the known strain of Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922) and incubated at 37°C overnight. After overnight incubation, the tubes are checked for the growth of the organisms and from the tubes sub cultures are made serially corresponding to the dilutions on to the Blood agar, Mac Conkey and Nutrient agar media, incubated at 37°C overnight. After overnight incubation, the dilution where the growth of the organisms does not occurred, are noted and tabulated. **RESULT:** The aqueous extract of Acacia catechu shows bactericidal effect up to the dilution of 1:8 whereas the aqueous extract of Ficus hispida shows no bactericidal effect even at higher concentration. **CONCLUSION:** These plants extract have been using for the treatment of ailments in the villages of the state of Manipur by the local quacks since time immemorial. In this study it has seen that Acacia catechu has the medicinal properties whereas Ficus hispida has no bactericidal property though the plant has other medicinal properties. It is therefore, necessary to do further studies to identify the ingredients present in this two plants for safe use and large scale production in the state.

**KEYWORDS:** Acacia catechu, Ficus hispida, Medicinal plant, Bactericidal, Soxhlated.

**INTRODUCTION:** A Vast majority of population particularly those living in villages consults traditional physicians (local quacks – in Manipuri they are called Maibas) for the treatment of diseases or for any ailments. The maibas on the other hand depend largely on herbal medicines for the treatment of such diseases or ailments.

These medicinal plants are grown in wild and available in plenty. The maibas used the plants in the form of extract, crushed juice etc obtained from the leaves, fruits, bark or roots of the plants that are given in divided doses. Among the plants, the species of Acacia, Fiscus, are some of the plants used as hypoglycemic, analgesic, diuretic, antipyretic, anti-inflammatory and bactericidal agents.

**ACACIA SPECIES** (Katha in Manipuri): Acacia catechu is a moderate sized tree and belongs to the family Leguminosai. It is 9-12 meter high having dark colored and rough bark with young shoots of

dark brown purple shade. It is found in Central India, Northeast Himalayas, Punjab, Bihar and Myanmar (Burma).

The bark of *Acacia catechu* is bitter and it was reported to have soothing and astringent activity in bowel and also anti-dysenteric, antidiarrhoea, antipyretic and antihelminthic effects.<sup>1</sup>

Chopra RN et al reported that *Acacia catechu* is found in Punjab, Northeast Himalaya, Bihar and throughout Konkan region. The bark and wood of it contains catechin, epicatechin, catechutannic acid and tannin.<sup>2</sup>

Cutch or catechu is an extract prepared from the hard wood *Acacia catechu*, occurs in nearly black masses, breaks easily and fractured surface have dull glossy look. It yields a dull powder, has no odour but has an astringent and sweetish taste. Catechu contains catechutannic acid (25.33%), acacatechin (10-12%), catechu red and guercetin.<sup>3</sup>

Nadkarni Km described that extract obtained from bark, wood, flowering tops and gum contains tannic acid 35%, catechuic acid or catechin, catechu red, tannin gum, guercetin and ash. It was used in diarrhea, haemorrhages, bedsores, cracked nipples, gonorrhoea, syphilis, leprosy, ulcer and gonorrhoeal joint pains. It was also used in dysentery, diarrhea, gonorrhoea, cystitis, vaginitis, diabetes mellitus and passive haemorrhage. *Acacia coccinea* was found to be useful in jaundice and in malaria and flatulence.<sup>4</sup>

**FICUS HISPIDA (Manipuri – Ashee heibong):** *Ficus hispida* (Manipuri – Ashee heibong) is a shrub or small tree found throughout the state of Manipur. It is used by Maibas (indigenous medicine man) in the treatment of diabetes mellitus.<sup>5</sup>

Sinha SC reported the therapeutic effect of *Ficus hispida* that are found throughout the state of Manipur growing up to 1500 meters above the mean sea level, in moist situation. Leaves are used in ringworm, latex on boils and fruits seeds in dysentery. Bark, fruit and seed are emetic and also used as purgative.<sup>6</sup>

Nadkarni KM described the medicinal uses of *Ficus hispida*. Bark is emetic and laxative. As powder or decoction (1 in 10), it is given in hepatic obstruction. Fruits and seeds act as antipyretic. A poultice of the bark is applied to the buboes. Figs of this plant promote the secretion of milk and preserved the fetus in the womb.

Various parts of this tree are effective in cases of Kusta, Vrana, Kapha, Pitta, Piles, Pandu and healer of wounds. In the treatment of Switra, the juice can be taken with jaggery as a Sramsana. A mixture of honey and the juice of this fruits prove a good anti-haemorrhagic. Powders of Hingu, the root of kapi-kachu with juice of the fruits, used as snuff is said to be a curative of Vatavyadhi. Root is said to cure poisons of Sarameya. Many uses are similar to *Ficus bengalensis*.<sup>4</sup>

**MATERIALS AND METHODS:** The two medicinal plants *Acacia catechu* (Katha in Manipuri) and *Ficus hispida* (Ashee heibong in Manipuri) were collected from different parts of Manipur during the period from March 2006 to February 2007 and processed in the laboratory to get the yield or extract as given below and also to determine the bactericidal effect on two known strains of *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922), as described in the Clinical Bacteriology by E. Joan Stokes.<sup>7</sup>

**i) ACACIA CATECHU (katha in Manipuri)**

**METHOD OF EXTRACTION:** 100gms of the dried bark powder available in the market was defatted with petroleum ether (40<sup>o</sup>c to 60<sup>o</sup>c) then extracted with ethanol (95%) in soxhlet apparatus. The active ethanolic extract was spread out to evaporate the adhering solvent and then extracted with ethyl acetate. The ethyl acetate was further concentrated and dried and stored in sterile porcelain jar. Then 600mg of the dried extract was dissolved in 1ml of distilled water to make a solution and proceed for the tests.<sup>8</sup>

**PROCEDURE:**

1. 6 (six) sterile test tubes containing 0.5 ml of Nutrient broth were arranged serially in two rows in the test tube rack, one row for each of the ATCC controls - Staphylococcus aureus and Escherichia coli.
2. 0.5 ml of the prepared plant extract solution was added to the first tube in each row and mixed thoroughly.
3. 0.5 ml of this mixture was transferred to the next tube, mixed thoroughly and again transferred 0.5 ml to the next tube and so on up to the 6<sup>th</sup> tube. The last 0.5 ml of the mixture from each row was discarded. Thus making a serial dilution of 1:1, 1:2, 1:4, 1:8, 1:16, and 1:32 of the plant extract for each row (as shown below)
4. 0.5 ml of the ATCC controls strains - Staphylococcus aureus and Escherichia coli prepared in normal saline matching with of 0.5 McFarland's turbidity were added to the respective tubes.
5. The tubes were incubated at 37<sup>o</sup>C overnight and results were interpreted as Minimal Inhibitory Concentration (MIC) of the drug in the dilution tubes of the rows in which turbidity and deposits were not observed.
6. From the dilution tubes subcultures were done on Blood Agar, Mac Conkey Agar and Nutrient Agar media for both the organisms.
7. The culture plates were incubated at 37<sup>o</sup>C for overnight.
8. The plate that showed no bacterial growth corresponding to the dilution tube was regarded as the Minimal Bactericidal Concentration (MBC). The results were recorded in the table form.
9. Two control tubes were also included in each row, one for known organism and the other for the extract containing 1ml each.

0.5 ml of Nutrient Broth & Extract + 0.5 ml of 0.5McFarland's turbidity of ATCC Staphylococcus/E. Coli strain.	1ml	1ml	1ml	1ml	1ml	1ml	Controls	
							Known orgm. (1ml).	Extract only (1ml).
Dilutions	1:1	1:2	1:4	1:8	1:16	1:32		
Dilution of the plant extract								

**ii) FICUS HISPIDA (Ashee heibong in Manipuri)**

**METHOD OF EXTRACTION:** 10 kg of bark of Ficus hispida were collected from different parts of the state of Manipur. Dried in the room temperature and powdered in the grinder. The powder form was defatted with petroleum ether (40<sup>o</sup>c to 60<sup>o</sup>c) then extracted with ethanol (95%) in soxhlet apparatus.

## ORIGINAL ARTICLE

---

The active ethanolic extract was spread out to evaporate the adhering solvent and then extracted with ethyl acetate. The ethyl acetate was further concentrated and dried and stored in sterile porcelain jar. Then 600mg of the dried extract was dissolved in 1ml of distilled water to make a solution and proceed for the test.<sup>8</sup>

Procedure of the dilution of the extract - Same as above.

**RESULT:** Bactericidal effect of aqueous extract of *Acacia catechu* showed inhibition of growth of both the organisms at 1:8 dilutions. The minimum inhibitory concentration (MIC) of both the organisms was recorded at 1:8 dilution of the extract and the minimum bactericidal concentration (MBC) was also noted at 1:8 dilutions. Table I and II.

Table III and IV shows that both organisms are grown in all the dilutions of the extract indicating that the extract of the *Ficus hispida* has no bactericidal effect on both *Staphylococcus aureus* and *Escherichia coli* strains.

**DISCUSSION:** In this study it was noted that the extract of the *Acacia catechu* showed the bactericidal effects up to the dilution of 1:8 on both the organisms i.e. the ATCC controls strains - *Staphylococcus aureus* and *Escherichia coli* whereas the extract of *Ficus hispida* showed no bactericidal effects even at higher concentration. Bently R and Trimen H reported that it contains catechin, catechuic acid, catechutannic acid and guercetin. They also reported that catechu is a valuable remedy in chronic diarrhea, dysentery and bleeding nose.<sup>9</sup>

Nadkarni Km described that extract obtained from bark, wood, flowering tops and gum contains tannic acid 35%, catechuic acid or catechin, catechu red, tannin gum, guercetin and ash. It was used in diarrhea, haemorrhages, bedsores, cracked nipples, gonorrhoea, syphilis, leprosy, ulcer and gonorrhoeal joint pains. It was also used in dysentery, diarrhea, gonorrhoea, cystitis, vaginitis, diabetes mellitus and passive haemorrhage. *Acacia cocinna* was found to be useful in jaundice and in malaria and flatulence.<sup>4</sup>

Sinha SC reported the therapeutic effect of *Ficus hispida* that leaves were used in ringworm, latex on boils and fruits seeds in dysentery. Bark, fruit and seed are emetic and also used as purgative.<sup>6</sup> In Manipur the local quacks (maibas) used these medicinal plants in the treatment of diarrhoea, dysentery and other ailments in the form of juice obtained from the leaves, barks and fruits and were given in divided doses for few days.

**CONCLUSION:** The aqueous extract from the bark of the *Acacia catechu* has the anti-bacterial properties. However, the exact chemical composition that leads to bactericidal effect on the two known strain of the organisms is not known. Further study is needed to identify this chemical compounds. If these compounds are found in plenty in the plant itself, large scale plantation may be encouraged. This may lead to the development of pharmaceutical industries in the state of Manipur which in turn will help to improve the socio-economic condition of Manipur.

The aqueous extract of *Ficus hispida* has no anti-bacterial/bactericidal property on the two known bacterial strains. Hence, its use by the local quacks in the treatment of diarrhea and dysentery should be restricted and the useful property of this medicinal plant should also be emphasized to the local quacks for proper use.

## ORIGINAL ARTICLE

### Bactericidal effect Acacia Catechu:

0.5 ml of *N.B & Extract +0.5ml of 0.5McFarland's turbidity of ATCC Staphylococcus	1ml	1ml	1ml	1ml	1ml	1ml	Controls	
							Known orgm. (1ml).	Extract only (1ml).
Dilutions	1:1	1:2	1:4	1:8	1:16	1:32		
MIC	No growth	No growth	No growth	No growth	Growth	Growth	Growth	No growth
MBC	No growth	No growth	No growth	No growth	Growth	Growth	Growth	No Growth

**Table I: Bactericidal effects of Acacia catechu on Staphylococcus aureus**

\*Nutrient Broth

Bactericidal effect at the dilution of 1:8

0.5 ml of *N.B & Extract +0.5ml of 0.5McFarland's turbidity of ATCC E. Coli strain.	1ml	1ml	1ml	1ml	1ml	1ml	Controls	
							Known orgm. (1ml).	Extract only (1ml).
Dilutions	1:1	1:2	1:4	1:8	1:16	1:32		
MIC	No growth	No growth	No growth	No growth	Growth	Growth	Growth	No growth
MBC	No growth	No growth	No growth	No growth	Growth	Growth	Growth	No Growth

**Table II: Bactericidal effects of Acacia catechu on Escherichia coli**

\* Nutrient Broth

Bactericidal effect at the dilution of 1:8

### d) Bactericidal effect of Ficus hispida:

0.5 ml of *N.B & Extract + 0.5ml of 0.5McFarland's turbidity of ATCC Staphy. strain	1ml	1ml	1ml	1ml	1ml	1ml	Controls	
							Known orgm. (1ml).	Extract only (1ml).
Dilutions	1:1	1:2	1:4	1:8	1:16	1:32		
MIC	Growth	Growth	Growth	Growth	Growth	Growth	Growth	No Growth
MBC	Growth	Growth	Growth	Growth	Growth	Growth	Growth	No Growth

**Table III: Bactericidal effects of Ficus hispida on Staphylococcus aureus**

\* Nutrient Broth

No Bactericidal effects.

## ORIGINAL ARTICLE

0.5ml of *N.B & Extract +0.5ml of 0.5McFarland's turbidity of ATCC Esch.Coli strain.	1ml	1ml	1ml	1ml	1ml	1ml	Controls	
							Known orgm. (1ml).	Extract only (1ml).
Dilutions	1:1	1:2	1:4	1:8	1:16	1:32		
MIC	Growth	Growth	Growth	Growth	Growth	Growth	Growth	No growth
MBC	Growth	Growth	Growth	Growth	Growth	Growth	Growth	No Growth

Table IV: Bactericidal effects of *Ficus hispida* on *Escherichia coli*

\* Nutrient Broth

No Bactericidal effect

### BIBLIOGRAPHY:

1. Kirtikar KR and Basu BD. *Acacia* (Tourn) Linn. In: *Indian Medicinal plants*. 2<sup>nd</sup> ed. Dehradun, International Book Distributors, Book sellers and Publishers; 1935: Vol 2; 919-935.
2. Chopra RN, Nayar SL and Chopra IC. *Eupatorium* (compositae). In: *Glossary of Indian Medicinal plants*. Council of Scientific and Industrial Research, New Delhi, 1956:133.
3. Wallis TE. *Cutch Text Book of Pharmacology*. 5<sup>th</sup> ed. London, J and A Churchill Ltd, 1967: 461-463.
4. Nadkarni KM. *Acacia catechu* Willd. In: *KM Nadkarni's Indian Materia Medica*. 2<sup>nd</sup> ed. Bombay, Popular Prakashan Pvt. Ltd; 1996: Vol 1, 11-13.
5. Khuman G. *Ashee heibong; Kangleipakki Hidak Pambi* (English: *Traditional medicinal plants of Manipur*), 1<sup>st</sup> ed. Imphal, Khuman G. Khuman Publishers; 1995: 41.
6. Sinha SC. *Ficus hispida* Linn. *Medicinal plants of Manipur*. 1<sup>st</sup> ed. Imphal, Manipur Association of Science and Society (MASS), 1996: 77.
7. E. Joan Stokes. *Clinical Bacteriology*. 4<sup>th</sup> ed. London, Edward Arnold Publishers Ltd, 1975: 253.
8. Jayasekhar P, Mohanan PV and Rathinam K. Hepatoprotective activity of ethyl Acetate of *acacia catechu*. *Indian J. Pharmacol.* 1997; 29: 426-428.
9. Bently R and Trimen H. *Acacia catechu* Wild, *Medicinal plants*. 2<sup>nd</sup> ed. London, J and A Churchill Ltd; 1980: 95.

**AUTHORS:**

1. H. Lokhendro Singh
2. S. Lokendrajit Singh
3. S. Rita Devi
4. Ng Brajachand Singh
5. L. Supriya

**PARTICULARS OF CONTRIBUTORS:**

1. Professor and HOD, Department of Microbiology, JNIMS, Porompat, Imphal East Manipur.
2. Junior Research Fellow, ISM, Department of Microbiology, RIMS, Imphal West Manipur.
3. Professor, Department of Pharmacology, RIMS, Imphal West, Manipur.
4. Professor, Department of Microbiology, RIMS, Imphal West, Manipur.

5. Assistant Professor, Department of Microbiology, JNIMS, Imphal East.

**NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR:**

Dr. P. Krishna Prasad,  
H. No. 64-1-4/4,  
Pratap Nagar,  
Kakinada-533001.  
Email: drkp79@gmail.com

Date of Submission: 05/07/2014.

Date of Peer Review: 07/07/2014.

Date of Acceptance: 22/07/2014.

Date of Publishing: 30/07/2014.