



Research Article

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF *CASSIA FISTULA* AND *BAUHINIA VARIEGATA*: A COMPARATIVE STUDY AND PHARMACOLOGICAL IMPORTANCE

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ABSTRACT

Present study was designed to evaluate the antibacterial and antifungal activities of two commonly available medicinal plants *Cassia fistula* (Amaltas) and *Bauhinia variegata* (Kachnar) leaf oil against the commonly pathogenic bacteria (*Proteus Vulgaris*, *Pseudomonas aeruginosa*, *Salmonella newport* and *Salmonella stanley*) and fungi (*Rhizopus stolnifer*, *Pencillium digitatum*, *Pencillium notatum* and *Aspergillus niger*). Leaf extract was by isolated steam distillation using Clevenger apparatus. The antibacterial activity was tested by paper disc diffusion method. Nutrient agar medium was used throughout the experiment for maintain the culture and also for testing. Penicillin and tetracycline were used for comparison of antibacterial activities. 5 ml of sterilize nutrient agar was poured in sterilized petri plates and allowed to cool under standard conditions. The Tubes were incubated with the bacteria at room temperature for 48 to 72 hours depending on the optimum growth of fungi. Mycostatin was used as standard for comparison of antifungal activities. Based on the results of the present findings it my suggested that leaf extract and its oil could used against the bacterial and fungal activities.

Keywords: Antibacterial, antifungal, leaf extract, leaf oil, *Cassia fistula*, *Bauhinia variegata*

INTRODUCTION

Since ancient time natural products, essentially plants have been used for the treatment of various diseases in India and abroad. Traditionally, in India, their various forms like powder, syrup etc. of plant products has been very frequently used for the remedy of the various diseases. The vegetative and reproductive parts of the plant are extensively used against a number of disorders research suggests primary and secondary metabolites of the plant products are mostly probably essential for their beneficial effects. Therefore medicinal plants play a vital role for the development of new drugs. Other countries like Egypt, china and Greece are also using terrestrial plants as medicine with an impressive result in the era of modern drug¹. The first available record the uses of plants as medicine was appeared approx. 2660 B.C. from the Sumerians and Akkaidians.

Traditional medicine is the synthesis of therapeutic experience of generation of practicing physicians of indigenous system of medicine. Among the remedies used, plant drugs constitute an important part, Asteraceae, Liliaceae, Apocynaceae, Solanaceae, Casalpinaeae, Rutaceae, Piperaeae, Sapotaceae, all these families have been found to use as medicinal plants by scientific investigator². In this connection, *Cassia fistula* Linn. and *Bauhinia variegata* are very common plants and is widely known for its medicinal properties.

The mountain ebony, *Bauhinia variegata* L. belongs to the family leguminosae. It is distributed throughout India, ascending to an altitude of 1300 in Himalayas. It is widely planted in the tropics and warm regions of the world (The wealth of India Raw

Material). More than 200 species of this genus are documented till date.

Bauhinia variegata is a medium sized deciduous tree. The genus includes trees, vines and shrubs that are frequently planted for their showy flowers and ornamental foliage. This plant may be used for many pharmacological properties. It is commonly known as Kachnar in Hindi, Sanskrit and mountain ebony in English³. The leaves are 10-15 cm long and broad, subcoriaceous and deeply cordate.

Casia fistula Linn. belongs to the family Caesalpiniaceae commonly known as Amaltas, Fistula, Laburnum, Purging Fistula, and Golden Shower in English popularly called “Indian Laburnum”. It is extensively used in various countries including Mauritius, India, South Africa, Mexico, China, West Indies, East Africa and Brazil as an ornamental tree for its beautiful bunches of yellow flowers. This plant has been described to be useful against skin diseases, liver difficulty, tuberculous glands and its use in the treatment of rheumatism, hematemesis, pruritus, leucoderma, diabetes and flowers and pods are used as a purgative, cough, retained excretions, biliousness⁴⁻⁶ (Alam, *et al.*, 1990; Asolkar, *et al.*, 1992 and Kirtikar and Basu, 2006).

The various parts of the plant in the traditional system of medicines are for the cure of diseases. In the present study leaves of *B. variegata* and *C. fistula*, to efficacy as a curative agent through pharmacological investigations. The aim of present study was to explore the traditional uses, pharmacognostical studies and pharmacological investigations which were carried out on the plants.

MATERIAL AND METHOD

Study of Antibacterial Activity

Cassia fistula (Amaltas) was collected from the periphery of Surguja District and identified by the taxonomist of department of Botany, Sri Sai Baba Aadrsh Mahaviyalayay, Ambikapur, Surguja District, C. G. Activity of plant parts of extract of *Cassia fistula* (Amaltas) was taken for the present investigation. Here the Leaf extract was by isolated steam distillation using Clevenger apparatus. The antibacterial activity was tested by paper disc diffusion method. The medium which is employed has the following composition.

Culture Media

The following "Nutrient agar medium" was used throughout the experiment for maintain the culture and also for testing.

NaCl – 5 g
Peptone – 20 g
Leaf extract – 20 g
Distilled water – 1000 ml

Preparation of Plant Extract

5 ml of sterilize nutrient agar was poured in sterilized petri plates and allowed to cool in standard position. The tubes were incubated with bacteria at room temperature for 40 to 72 hours depending on the optimum growth of fungi.

Test Organisms

The following bacteria were used for investigated

1. *Proteus vulgaris*
2. *Pseudomonas aeruginosa*
3. *Salmonella newport*
4. *Salmonella stanley*

Standard

Penicillin and tetracycline were used for comparison.

Study of Antifungal Activity

The antifungal activity of Amaltas was tested by paper disc diffusion method. The medium which is employed has the following composition.

Culture Media

The following "Sabaroud's broth" medium was used throughout the experiment for testing.

Pepton - 10 g
Glucose - 20 g
Distilled water – 1000 ml

Preparation of Plant Material

5 ml of sterilized sabaroud's agar was poured in sterilized petri plates and allowed to cool in standard position. The tubes were incubated with the fungi at room temperature to 24 to 48 hours depending on the optimum growth of fungi.

Test Organisms

The following fungi were used for investigated

1. *Rhizopus stolonifer*
2. *Pencillium digitatum*

3. *Pencillium notatum*

4. *Aspergillus niger*

Standard

Mycostatin was used for comparison.

Study of Antibacterial Activity

Bauhinia variegata (Kachnar) was collected from the periphery of Surguja District and identified by the taxonomist of department of botany, Sri Sai Baba Aadrsh Mahaviyalayay, Ambikapur, Surguja District, C. G. Activity of plant parts of extract of *Bauhinia variegata* (Kachnar) was taken for the present investigation. Here the leaves extract was by isolated steam distillation using Clevenger apparatus. The antibacterial activity was tested by paper disc diffusion method. The medium which is employed has the following composition.

Culture Media

The following "Nutrient agar medium" was used throughout the experiment for maintaining the culture and also for testing.

NaCl – 5 g
Peptone -20 g
Leaf extract – 20 g
Distilled water – 100 ml

Preparation of Plant Materials

5 ml of sterilize nutrient agar was poured in sterilized petri plates and allowed to cool in standard position. The Tubes were incubated with the bacteria at room temperature for 48 to 72 hours depending on the optimum growth of fungi.

Test Organism

The following were used for investigated

1. *Proteus vulgaris*
2. *Pseudomonas aeruginosa*
3. *Salmonella newport*
4. *Salmonella Stanley*

Standard

Penicillin and tetracycline were used for comparison.

Study of antifungal activity

The antifungal activity of Kachnar was tested by paper disc diffusion method. The medium which is employed has the following composition.

Culture Media

The following "Sabaroud broth" medium was used throughout the experiment for maintaining the culture and also for testing.

Peptone – 10 g
Glucose - 20 g
Agar – 10 g
Distilled water – 1000 ml

Preparation of Plant Materials

5 ml of sterilize sabaroud agar was poured in sterilized petri plates and allowed to cool in standard position. The Tubes were

incubated with the fungi at room temperature for 24 to 48 hours depending on the optimum growth of fungi.

Test Organism

The following were investigated

1. *Rhizopus stolonifer*
2. *Pencillium digitatum*
3. *Pencillium notatum*
4. *Aspergillus niger*

Standard

Mycostatin was used for comparison.

RESULTS

Determination of Antibacterial Activity of *Cassia fistula*

The antibacterial activity was tested for pure extract and at various dilutions using ethylene glycol as solvent at concentration of 5 ml/mg of phosphate buffer saline (w/v). The activity was studied by filter paper disc method. These were soaked with various samples to be tested and were dried at 50°C. The zone of inhibition was recorded at 36. After 48 hours. The various observation of plant parts extract of *Cassia fistula* (amalatas) is given in the Table.

Table 1: Antibacterial activity of the essential oil from the leaves of *Cassia fistula* (Amalatas)

S. No.	Bacteria	Diameter of zone of inhibition (mm)			
		Leaves extract	Dilution		
			1:5	1:10	1:15
1	<i>Proteus vulgaris</i>	1.5	1	0	0
2	<i>Pseudomonas aeruginosa</i>	6.0	5	4.5	3.5
3	<i>Salmonella newport</i>	6.0	4.5	3.0	2.5
4	<i>Salmonella stanley</i>	2.0	2	1.5	0

Values are the average of four determinations in four different directions. Whatman 41 (6 mm) discs were soaked with each sample tested at concentration of mg/ ml of phosphate buffer saline (w/v). The dilution is done in ethylene glycol

Table 2: Minimum inhibitory concentration of essential oil from the leaves of *Cassia fistula*

S. No.	Bacteria	Minimum inhibitory concentration		
		Penicillin (x 10 mg/ml)	Tetracycline (x 10 mg/ml)	Essential oil (mg/ml)
1	<i>Proteus vulgaris</i>	0.18	0.3	7.5
2	<i>Pseudomonas aeruginosa</i>	0	0	1.5
3	<i>Salmonella newport</i>	0.15	0.2	2.0
4	<i>Salmonella stanley</i>	0.16	0.3	7.0

Table 3: Showing antifungal activity of *Cassia fistula*

S. No.	Fungi	Diameter of zone of inhibition (mm)				Mycostatin (10 g/ml)
		Essential oil	Dilution			
			1:5	1:10	1:15	
1	<i>Rhizopus stolnifer</i>	7.5	6.0	5.5	5.0	1.0
2	<i>Penicillium digitatum</i>	5.0	4.5	3.5	2.0	9.
3	<i>Pencillium notatum</i>	6.5	6.0	5.0	4.5	7.0
4	<i>Aspergillus niger</i>	5.5	5.0	4.5	4.0	7.0

Values are the average of four determinations in four different directions. Whatman 4.1 (6 mm) discs were soaked with each sample tested at a concentration of 6 mg/ml of phosphate buffered saline (w/v). Here also dilution was done by ethylene glycol

Table 4: Antibacterial activity of the essential oil from of *Bauhinia variegata* (Kachnar)

S. No.	Bacteria	Diameter of zone of inhibition (mm)			
		Essential Oil	Dilution		
			1:5	1:10	1:15
1	<i>Proteus vulgaris</i>	3.0	2.5	2.0	1.5
2	<i>Pseudomonas aeruginosa</i>	4.0	3.5	2.5	1.0
3	<i>Salmonella newport</i>	8.0	7.5	6.5	5.5
4	<i>Salmonella stanley</i>	6.5	5.0	4.5	4.0

Values are the average of four determinations in four different directions. Whatman 41 (6 mm) discs were soaked with each sample tested at concentration of 6 mg/ of phosphate buffer saline (w/v). The dilution is done in ethylene glycol

Table 5: Minimum inhibitory concentration of essential oil from the leaves of *Bauhinia variegata*

S. No.	Bacteria	Minimum inhibitory concentration		
		Penicillin (x10 mg/ml)	Tetracycline (x10 mg/ml)	Essential oil (mg/ml)
1	<i>Proteus vulgaris</i>	3.0	2.5	2.0
2	<i>Pseudomonas aeruginosa</i>	4.0	3.5	2.5
3	<i>Salmonella newport</i>	8.0	7.5	6.5
4	<i>Salmonella stanley</i>	6.5	5.0	4.5

Table 6: Antifungal activity of leaf oil of *Bauhinia variegata*

S. No.	Fungi	Diameter of zone of inhibition (mm)			Mycostatin (10 g/ml)	
		Essential Oil	Dilution			
			1:5	1:10		1:15
1	<i>Rhizopus stolonifer</i>	4.5	7.0	6.5	6.0	4.0
2	<i>Penicillium digitatum</i>	5.5	5.0	4.5	3.0	6.0
3	<i>Penicillium notatum</i>	8.5	7.5	6.5	6.0	9.0
4	<i>Aspergillus niger</i>	8.5	8.	7.0	6.5	3.5

Values are the average of four determinations in four different directions. Whatman 41 (6 mm) discs were soaked with each sample tested at concentration of 6 mg/ of phosphate buffer saline (w/v). The dilution is done in ethylene glycol

Determination of Antifungal Activity *Bauhinia variegata*

The antifungal activity was tested for leaves extract and at various dilutions using ethylene glycol as solvent at a concentration of 5 ml/mg of phosphate buffer saline (w/v). The activity was studied by filter paper disc method. These were soaked with various samples to be tested and were dried at 40°C. The zone of inhibition was expressed as average of maximum dimension in 4 different directions.

Determination of Antifungal Activity of *Cassia fistula*

The antifungal activity was tested for leaves extract and at various dilutions using ethyleneglycol as solvent at a concentration of 5 mg/ml of phosphate buffer saline (w/v). The activity was studied by filter paper disc method. These were soaked with various samples to be tested and were dried at 40°C. The zone of inhibitions was expressed as average of maximum dimension in four different directions.

Determination of Antibacterial Activity of *Bauhinia variegata* (Kachnar)

The antibacterial activity was tested for pure extract and at various dilutions using ethylene glycol as solvent at a concentration of 5 ml/mg of phosphate buffer saline (w/v). The activity was studied by filter paper disc method. These were soaked with various samples to be tested and were dried at 50°C. The zone of inhibition was recorded at 36.C after 48 hours. The Various observation of plant parts extract of *Bauhinia variegata* (Kachnar) is shown in given tables.

DISCUSSION

Antibacterial and antifungal agents remain the basis in the treatment of bacterial and fungal infections. However, from last decades, different products are being randomly used for the treatment of different infection, but appearance of drug resistance bacteria has become a challenge for medical sciences. Therefore, it necessitates developing new drugs for bacterial and fungal diseases. Use of plant products for the treatment of human and other animal diseases has some advantages such as bio degradability, availability, low toxicity and cost effectiveness. In the present study two commonly are investigated for their antibacterial and antifungal activities.

In the current study, leaf extract of *Cassia fistula* showed strong activity against the tested bacterial and oil extracted from leaves were used against the different fungal strains. The results were compared with standard antibiotic drugs. The oil from the leaf extract of *Cassia fistula* exhibited antibacterial activists against *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella newport* and *Salmonella stanley*. The MIC of the oil from the leaves for testing four different bacterial strains were used and *Penicillium notatum* was taken as standard. The leaf extract showed antibacterial activity, but significantly higher activity was

observed in *Salmonella newport* bacterial strain as shown in the Table 1. The minimum inhibitory concentration was also found in the same bacterial strain that was 0.15. The essential oil from the leaves of *Cassia fistula* was found to be active against *Pseudomonas aeruginosa*, *Salmonella Newport* and to retain its activity even at dilution of 1:15.

The oil extracted from Leaf of *Cassia fistula* showed antifungal activity when compared with the standard. The results strongly confirmed that plant have important compounds that can be used for the treatment of different fungal infections. Present study focused on the antifungal effect of extracted leaf oil and was used to check against different fungal strains such as *Rhizopus stolonifer*, *Penicillium digitatum*, *Penicillium notatum* and *Aspergillus niger*. The resulted were supported by previous findings⁷. The essential oil was found to be active against *Penicillium naotatum* and *Rhizopus stolonifer* and to retain the activity even at a dilution of 1:15

The results of the present study provide evidences indicating strong antibacterial activity leaf extract *Bauhinia variegata*. The MIC leaf extract further, reveals the potential against the *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella newport* and *Salmonella stanley* bacterial strains. The antibacterial activity of the leaf may be proposed due to the presence of different bioactive components present in the leaves such as phenols, tannins, flavonoids, alkaloids and cardiac glycosides⁷. However, it is reported that it possesses some medicinal significance against the different bacterial infections⁹. The leaf of *Bauhinia variegata* was found to be active against *Salmonella satnley*, *Salmonella newport* and to retain its activity even at a dilution of 1:15.

The oil extract from the leaves were tested for antifungal activity against the different fungal strains such *Rhizopus stolonifer*, *Penicillium digitatum*, *Penicillium notatum* and *Aspergillus niger*. The antifungal activity was compared with standard Mycostatin (10 mg/ml). The table showed the results of antifungal activity of leaf oil of *Bauhinia variegata*. The leaf oil of *B. variegata* showed strong inhibitory activity against the *Rhizopus stolonifer*, *Penicillium digitatum*, *Penicillium notatum* and *Aspergillus niger*. This study confirms minimum inhibition zone of that leaf oil showed against all the fungal strains. Hence, leaf oil of *B. variegata* could be used as potential source of new antibiotics against the pathogenic fungi. The results of the present study are in agreement with previous findings¹⁰, suggesting antifungal activities against the different fungal strains of whole plant (leaves root and stem). The essential oil was found to be active against of *Aspergillus niger* and *Penicillium notatum* and to retain its activity even at a dilution of 1:15.

CONCLUSION

Numerous modern drugs till date have been isolated from the *C. fistula* as natural pool of therapeutic drugs to make the drugs free from side effects observed by many other synthetic/ non-herbal products. Nevertheless since ancient times especially in India

almost all diseases were treated or managed by the natural products. We may infer from the present study that *C. fistula* and *B. variegata* are rarely important considering their potential medicinal effects. Therefore the extract of leaf can be suggested to be utilized for the pharmaceutical formulation, however their action which can be helpful for the purpose of therapeutic potential need further exploration to know the leading molecule in search of new herbal drug.

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