Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2010, 2(6): 73-83
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X

Antimicrobial testing of the extracts of *Samanea saman* (Jacq.)Merr

P. Arulpriya[1], P. Lalitha[2] and S. Hemalatha[3]

Department of Chemistry, Avinashilingam Deemed University for Women, Coimbatore

ABSTRACT

The plant extracts of Samanea saman (Jacq.)Merr and its fractionated portions were examined for their antibacterial and antifungal activity. Two bacteria- Escherichia Coli, staphylococcus aureus and two fungi Aspergillus flavus, Candida albicans were used for the studies. The extracts were found to exhibit appreciable antimicrobial activity.

Key word: *Samanea saman(Jacq.)Merr,* Antibacterial activity, Antifungal activity.

INTRODUCTION

Samanea saman (rain tree) is a tropically found common plant which mitigates multitude of diseases and ailments [1]. Rain tree is a folk remedy for colds, diarrhoea, headache, intestinal ailments, and stomach ache [2]. There are several folk remedies prepared from various parts of rain tree. The root decoction is used in hot baths for stomach cancer [3].

Many higher plants accumulated extractable organic substances in quantities sufficient to be economically useful as pharmaceuticals/antibiotics [4]. Plants have been a rich source of medicines because they produce wide array of bioactive molecules, most of which probably evolved as chemical defense against predation or infection [5].

In many developing countries traditional medicine is one of the primary health care systems [6, 7]. India is well known for Ayurveda, which is one of important traditional medicine practiced. Herbs are widely exploited in the traditional medicine and their curative potentials are well documented [8]. Alternatives to available antibiotics for disease management are increasingly felt due to the increase in the resistance of bacterial isolates. Antibacterial and antifungal active principles isolated from higher plants is appears to be one of the important alternative approaches to contain antibiotic resistance and the management of disease. It is believed that plant based drugs cause less or no side effect when compared with synthetic antibiotics [9].

MATERIALS AND METHODS

Collection of plant

Combination of dried fallen leaves, flowers and stems of *Samanea saman* (Jacq.) Merr. were collected from Coimbatore.

Preparation of extracts

Solvent extracts (Petroleum ether, ethyl acetate, chloroform, Aqueous and HCl) were prepared by refluxing 940g of the fallen parts of *Samanea saman* with 1.5 liter of the appropriate solvent. The petroleum ether, ethyl acetate chloroform and aqueous extracts of *Samanea saman* obtained by conventional refluxing with corresponding solvents were chemically fractionated using HCl, dichloromethane (DCM), hexane (HEX), and acetone (ACT).

Activity studies

The plant extract and its fractions were tested for their antibacterial and antifungal activity. Two bacteria- *Escherichia Coli, staphylococcus aureus* and two fungai *Aspergillus flavus, Candida albicans* were used for the studies.

Preparation of culture media

SDA medium (Sabouard Dextrose Ager)

Contents	g/liter
SDA	65g
Distilled water	100ml

Suspended 65.0g of SDA in 100ml distilled water and boiled to dissolve the medium

Muller-Hinton Medium

Contents	g/liter
Acid Casein Peptone (H)	17.50g
Starch	1.50g
Beef Infusion	2g
Bacteriological Agar	17g

Suspended 38 g of the medium in one liter of purified water. Heated with frequent agitation and boiled for one minute to completely dissolve the medium. Autoclaved at 121°C for 15 minutes and cooled to room temperature.

Antimicrobial Testing

Disc Method for Determination of Zone of Inhibition for Antibacterial l activity

Paper discs of 4 mm diameter and glass Petri plates of 90 mm diameter were used throughout the experiment. Paper discs were sterilized in an autoclave and dried at 100°C in an oven. Then the discs were soaked with test chemicals at the rate of 50 µg (dry weight) per disc for antibacterial analysis. One drop of bacterial suspension was taken in a sterile Petri dish and then approximately 20 ml of sterilized and melted nutrient agar (~45°C) was poured into the plate, and then mixed thoroughly.

The paper discs after soaking with test chemicals were placed at the center of the inoculated pour plate. A control plate was also maintained in each case with alcohol. First, the plates were maintained at low temperature (4°C) for 4 hours. The plates were then incubated at (35 ± 2) °C for growth of test organisms and were observed at 24 hour intervals for two days. The activity was expressed in terms of zone of inhibition in mm. Each experiment was repeated thrice. The

standard antibiotics gentamycin were used as a positive control and compared with test chemicals under identical conditions.

Streak Plate Isolation Method for Determination of Zone of Inhibition for Antifungal Activity

The required amount of SDA medium was taken in a conical flask separately and was sterilized in autoclave (at 121°C and 15 Psi) for 15 min. A tube of SDA was liquefied and poured into the Petri dish. The plate was rotated gently for uniform distribution of the medium. The inoculating loop was held at a 60°C angle in the hottest part of the Bunsen burner flame. The entire tube was heated to redness. The loop was allowed to cool for 15 to 20 seconds before it touches the culture. A small amount of the culture was taken from the tube with a sterilized inoculating loop and the microorganisms were streaked in a plate following quadrant. The stock solutions were prepared following the quadrant by dissolving the compounds in ethanol.

The process of inoculation was done under aseptic condition and the spores were inoculated in the medium and incubated for 5 days. A clear zone or ring on the SDA plate develops, the diameter of which is measured as the zone of inhibition. The antimicrobial activities of the compounds were recorded by photographing the Petri dishes.

RESULTS AND DISCUSSION

Antimicrobial Studies of the Extracts of *Samanea Saman*

The solvent extracts of *Samanea saman* and its fractions were screened for antibacterial and antifungal activity against two bacteria- *Escherichia Coli, Staphylococcus aureus* and two fungi *Aspergillus flavus, Candida albicans*. The results of the antimicrobial studies of the extracts are given in **Table 1**.

Antibacterial Activity

The *Samanea saman* extracts and its fractions were screened for their antibacterial activity against *Escherichia coli* and *Staphyloccus aureus* by disc diffusion method using isopropanol as solvent. 50µg/ml solutions of extracts were compared with standard drug gentamycin. A clear zone of growth inhibition was noted around the disc due to diffusion of drug. **Plates 1-12** show the antimicrobial activity of compounds. The diameter of the inhibition zone denotes the relative susceptibility of the test micro organisms to a particular anti microbe.

Zone of Inhibition (mm)	Type of antimicrobe
> 13	Highly sensitive or susceptible
8-13	Moderately sensitive or intermediate
< 8	Resistant

It is seen from the results that among the fractionated portion, the P.DCM fraction showed good activity or is highly sensitive to *E.Coli*. The P.HEX and P.ACT fraction were found to be resistant against *E.Coli*. The E.DCM, E.HEX and E.ACT fractionated portion were found to be moderately sensitive against *E.Coli*. These fractionates were found to show half the activity of the standard gentamycin. The CH.DCM fraction showed good activity or is highly susceptible to *E.Coli*. The CH.HEX and CH.ACT fraction were found to be resistant against *E.Coli*.

The P.DCM fraction showed good activities susceptible to *Staphylococcus*. These fractionated portions were found to be show near activity of the standard gentamycin. P.ACT fractionated portion were found to be moderately sensitive. The P.HEX fractionated portions were found to

be resistant against *Staphylococcus*. The E.DCM, E.HEX and E.ACT fractionated portion were found to be moderately sensitive against *Staphylococcus*. The CH.DCM fraction showed good activity or is highly sensitive to *Staphylococcus*.

Table 1: Zone Of Inhibition Examined Against Two Bacteria and Two Fungi with the Extracts Of *Samanea saman* and its Fractionates

Extracts	Zone of Inhibition (mm) against		Zone of Inhibition (mm) against	
	Escherichia Coli	*Staphylococcus Aureus*	*Aspergillus Flavus*	*Candida Albicans*
PE	15	15	15	13
P.DCM	15	17	15	15
P.HEX	Resistant	3	Resistant	5
P.ACT	Resistant	10	Resistant	Resistant
EA	13.9	12	10.5	15
E.DCM	13	13.5	20	14
E.HEX	9	10	15.5	Resistant
E.ACT	8	9	13	Resistant
CH	12.1	16	8	12
CH.DCM	13.7	14	Resistant	10
CH.HEX	6	11	Resistant	Resistant
CH.ACT	5	10	Resistant	7
Aq	14.5	16	14	13
Gentamycin	20mm	Flucanozole	21mm	

Plate 1: Antibacterial activity of SS extracts against *E.Coli*

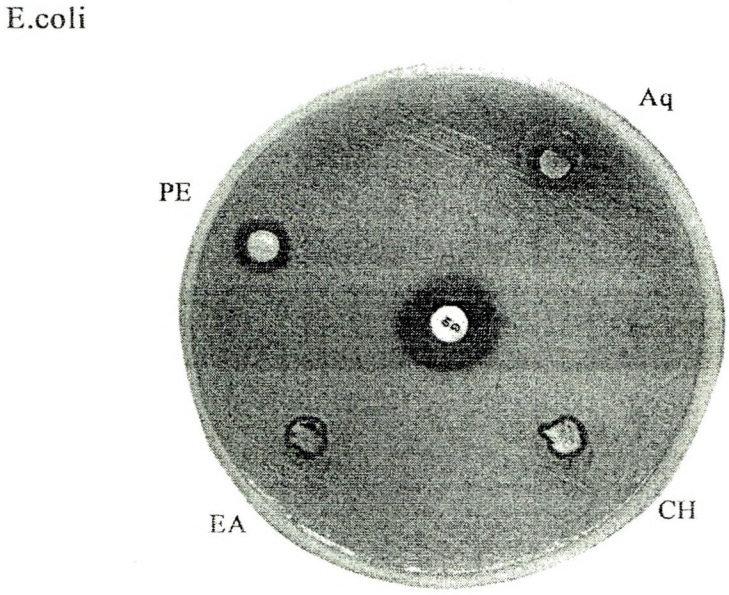


Plate 2: Antibacterial activity of SS extracts against *Staphylococcus aureus*

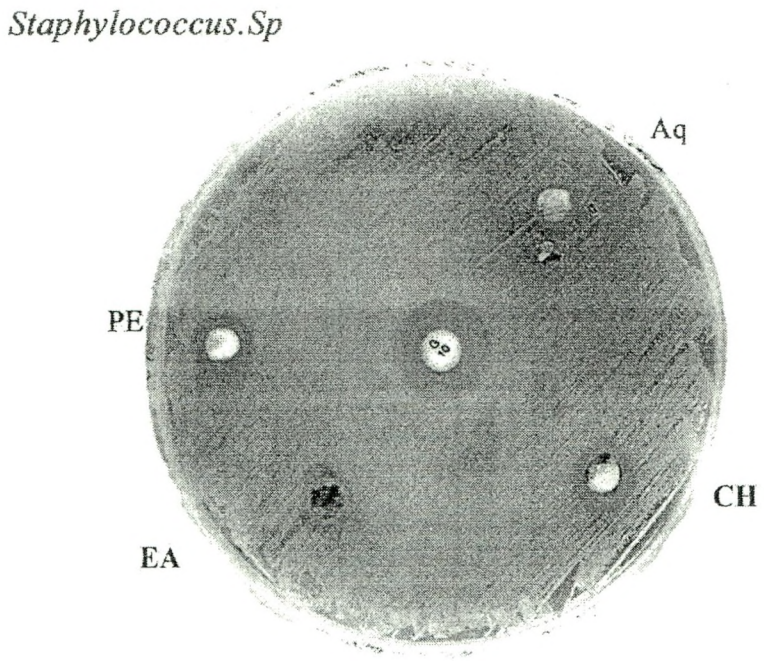


Plate 3: Antifungal activity of SS extracts against *Aspergillus niger*

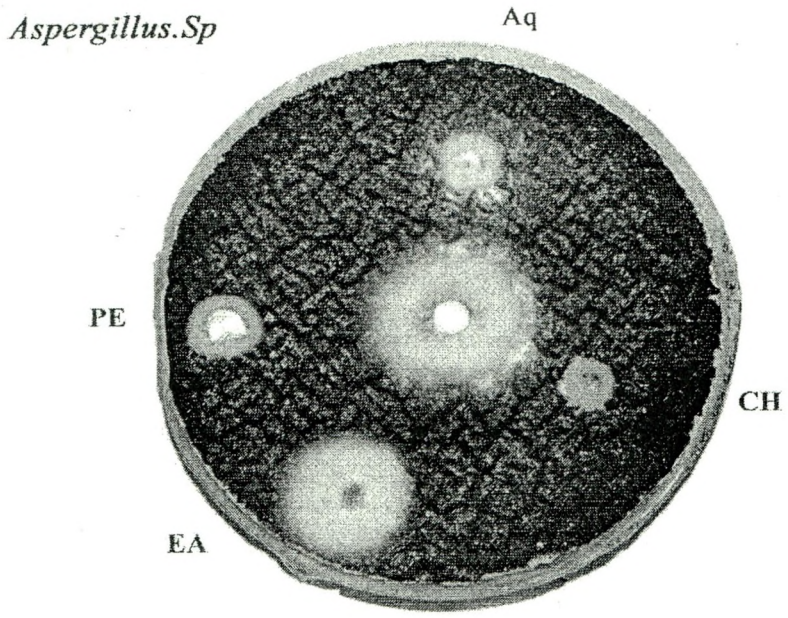


Plate 4: Antifungal activity of SS extracts against *Candida albicans*

Candida.Sp

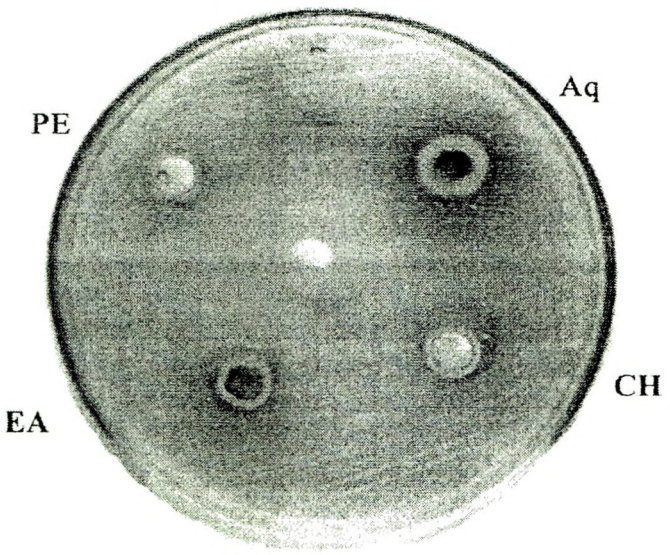


Plate 5: Antibacterial activity of EA extract and its fractionates against *E.Coli*

E.Coli

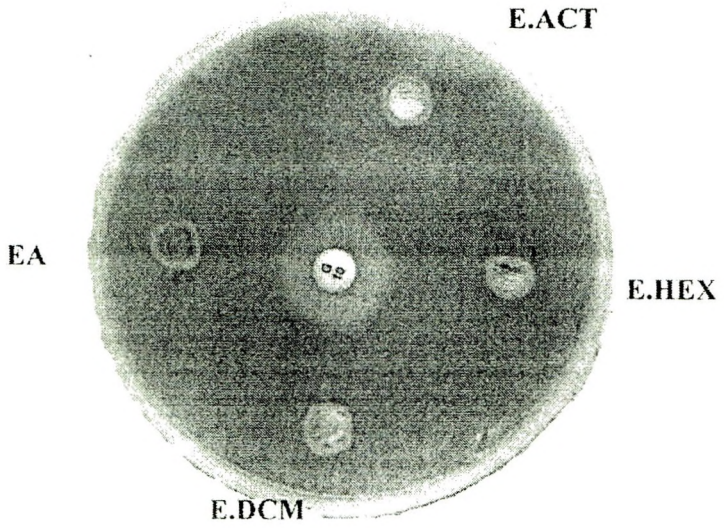


Plate 6: Antibacterial activity of EA extract and its fractionates against *Staphylococcus aureus*

Staphylococcus.Sp

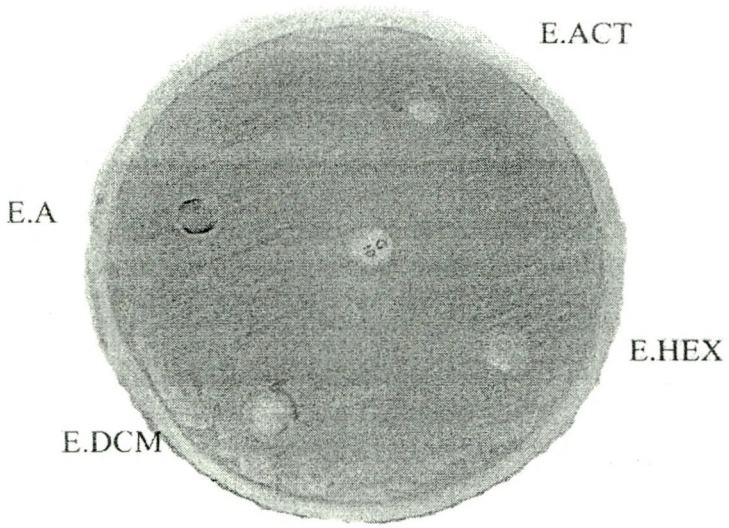


Plate 7: Antifungal activity of EA extract and its fractionates against *Aspergillus niger*

Aspergillus.Sp

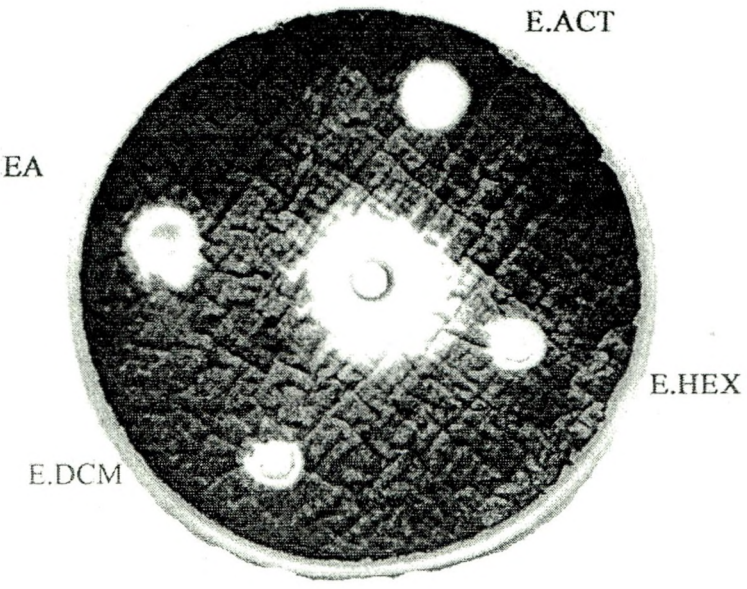


Plate 8: Antifungal activity of EA extract and its fractionates against *Candida albicans*

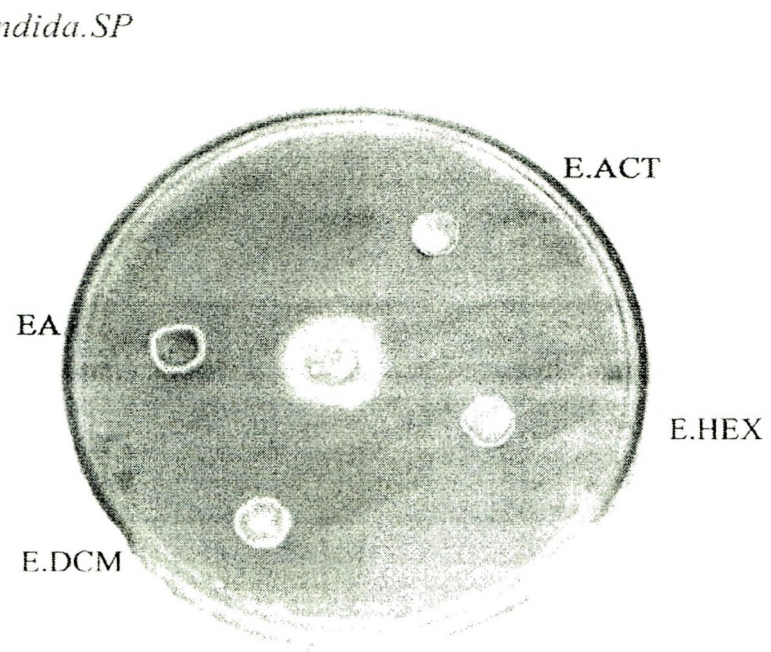


Plate 9: Antibacterial activity of PE and CH extract fractionates against *E.Coli*

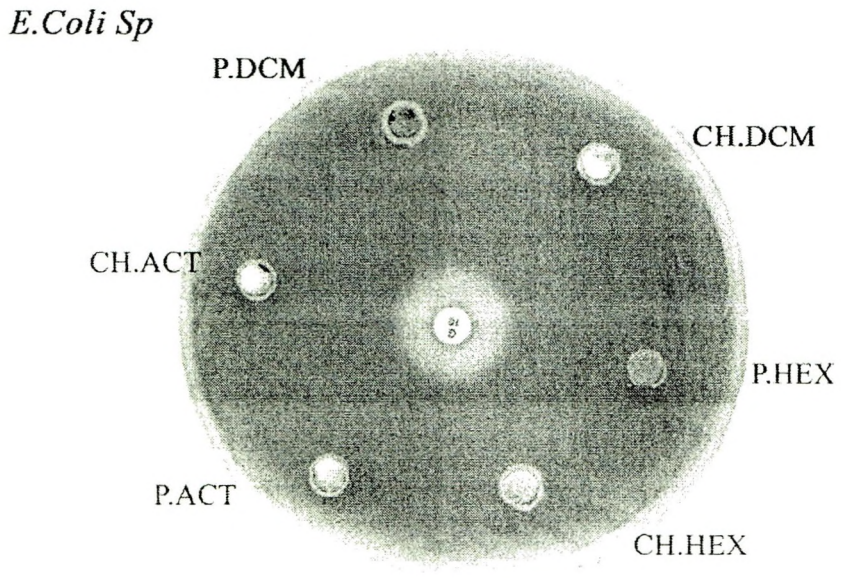


Plate 10: Antibacterial activity of PE and CH extract fractionates against *Staphylococcus aureus*

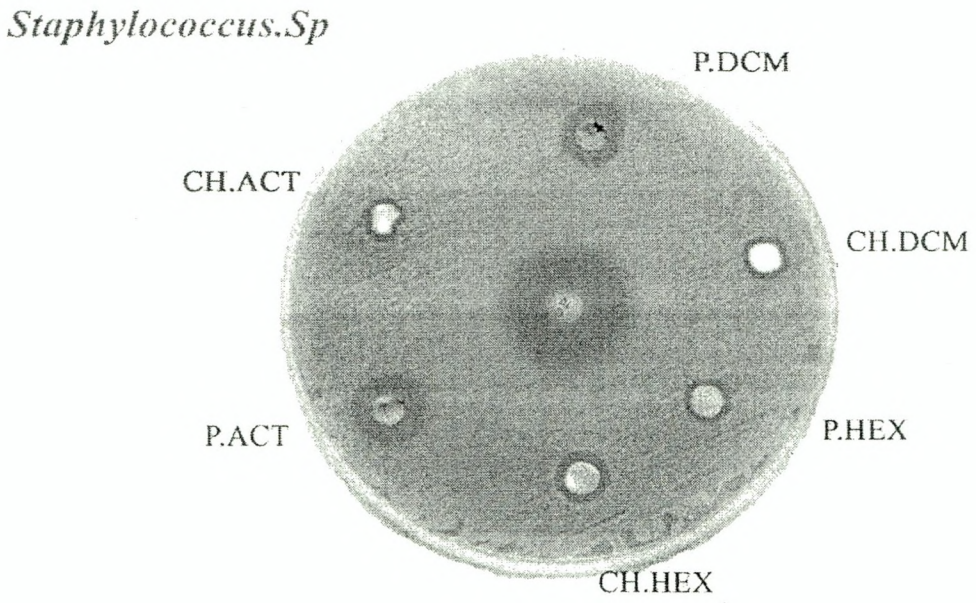


Plate 11 : Antifungal activity of PE and CH extracts fractionates against *Aspergillus niger*

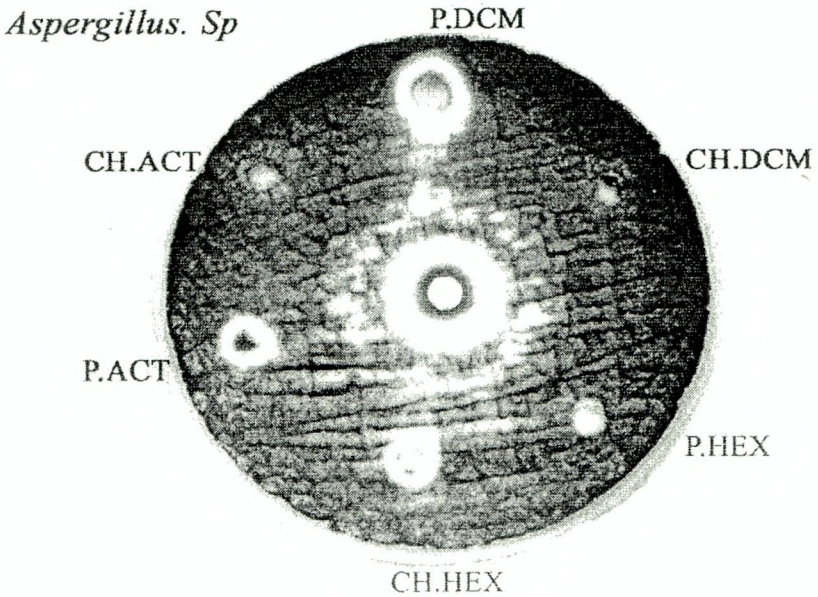
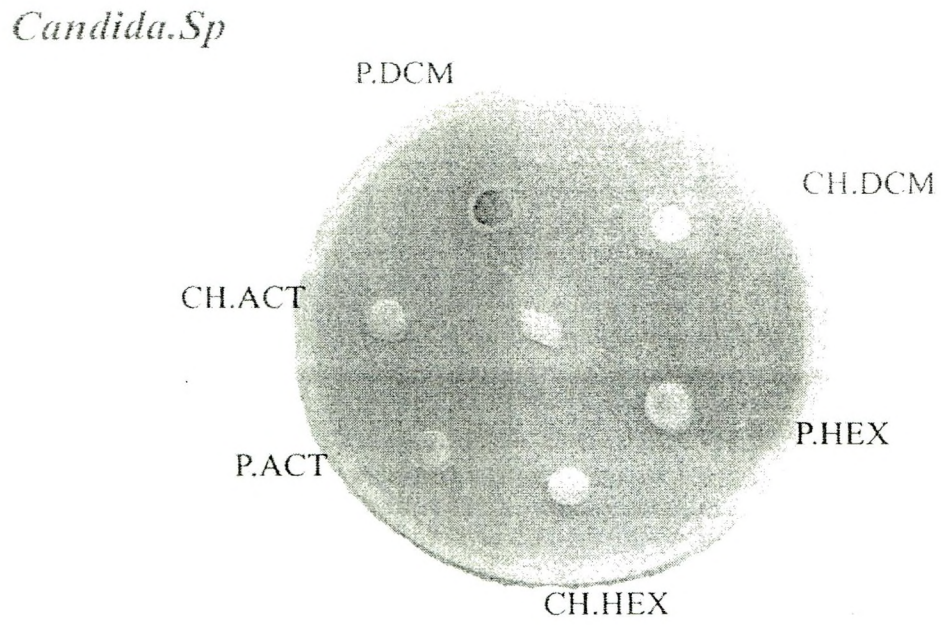


Plate 12 : Antifungal activity of PE and CH extracts fractionates against *Candida albicans*



The CH.HEX and CH.ACT fraction were found to be moderately sensitive against *Staphylococcus*. The synergistic effect of the extracts might be the reason for higher antibacterial activity compared to the fractions.

Antifungal Activity

The *Samanea saman* extracts and its fractions were screened for their antifungal activity against *Aspergillus flavus* and *Candida albicans* by streak plate isolation method using isopropanol as a solvent. A 50µg/ml solution of extracts was compared with standard drug flucanozole. Zone of inhibition are given in the **Table 1**. The P.DCM fraction showed good activities susceptible to *Aspergillus*. These fractionated portions were found to be shows near activity of the standard flucanozole. P.ACT fractionated portion were found to be moderately sensitive. The P.HEX fractionated portions were found to be resistant against *Aspergillus*.

The E.DCM, E.HEX and E.ACT fractionated portion were found to be moderately sensitive against *Aspergillus*. The CH.DCM fraction showed good activity or in highly sensitive susceptible to *Aspergillus*. The CH.HEX and CH.ACT fraction were found to be resistant against *Aspergillus*.

The P.DCM fraction showed good activities susceptible to *Candida*. The P.HEX and P.ACT fractionates were found to be resistant against *Candida*. The E.DCM fraction showed good activities susceptible to *Candida*. The E.HEX and E.ACT fractionated portions were found to be resistant against *Candida*. The CH.DCM fraction showed moderate sensitive susceptible to *Candida*. These fractionates were found to show half the activity of the standard flucanozole. The CH.HEX and CH.ACT fraction were found to be resistant against *Candida*. The synergistic effect of the extracts might be the reason for higher antifungal activity compared to the fractionates.

CONCLUSION

Results of antibacterial study of PE extract showed significant activity against *E.Coli*, where as Aq and CH extract showed significant activity against *Staphylococcus*. The antibacterial activities of the extracts were comparable with that of standard gentamycin and also results of antifungal study of PE extracts showed significant activity against *Aspergillus*, whereas the Aq extract showed significant activity against *Candida*. The antifungal activities of the extracts were comparable with that of standard flucanozole

Acknowledgement
The authors thank the authorities of the Avinashilingam Deemed University for Women, Coimbatore-43 for having providing the facilities to carry out this research work.

REFERENCES

[1] W.George Staples, R.Craig, Elevitch, Species Profiles for Pacific Island Agro orestry, **2006**, (www.traditionaltree.org).
[2] J.A .Duke, K.K. Wain, Medicinal plants of the world, **1981**, 3.
[3] J.L. Hartwell, Lloydia, **1967–1971**, 30–34.
[4] M.F. Balandrin, J.A. Klocke, E.S. Wurtele, W.H. Bollinger, *Science.*, **1985**. 228: 1154-1160.
[5] P.A .Cox, M.J. Balick, *Sci. Amer.*, **1994**, 270: 60-65.
[6] N.R.Fransworth, *J. Ethnopharmacol.*, **1993**, 38: 45-152.
[7] P.J.Houghton, *J. Altern and Complement Med.*, **1995**, 1: 131-143.
[8] N.K. Dubey, R. Kumar, P. Tripathi, *Curr. Sci.*, **2004**, 86: 37-41.
[9] N.Shariff, M.S. Sudarshana, S. Umesha, P. Hariprasad, *Afr. J. Biotechnol.*, **2006**, 5: 946-950.