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"STUDY OF IN-VITRO ANTI-BACTERIAL ACTIVITY OF *FLUEGGEA LEUCOPYRUS* LEAVES EXTRACT BY USING ETHANOL SOLVENT"

V. Gnanambiga[*1], S.Venkateshwaran [*2], T. Pattabiraja[*3] , G. Logesh[*4] ,M. Sathya[*5]

[*1] Associate professor, Department of pharmacognosy, Sri Vijay Vidyalaya College of Pharmacy, Dharmapuri, Tamilnadu, India.

[*2] Associate professor, Head of the department of pharmacognosy, Sri Vijay Vidyalaya College of Pharmacy, Dharmapuri, Tamilnadu, India.

[*3, *4*,*5,] B.Pharm students, Sri Vijay Vidyalaya College of Pharmacy, Dharmapuri, Tamilnadu, India.

Abstract:

Title: *"Study of In-vitro anti-microbial activity of Flueggea leucopyrus Leaves extracts by using ethanol solvents"*

Objective: *To the antimicrobial activity of flueggea leucopyrus leaves extracts.*

Method: *The leaf was collected and shade dried, made into coarse powder. The powdered plant material was subjected to successive solvent extraction by Soxhlet extraction method using ethanol as a solvent. The further extract was determined by preliminary phytochemical and antibacterial activity*

Result: *Shows the antibacterial activity of ethanolic extract of flueggea leucopyrus leaves and identified the phytochemicals.*

Conclusion: *A better results found in plant extract and providing against E. coli, staphylococcus aureus, Candida albicans and klebsiella pneumoniae.*

Keywords: *Flueggea leucopyrus, Soxhlet method, Phytochemical screening, Antibacterial activity.*

Corresponding author:

V. Gnanambiga,

Associate professor, Department of pharmacognosy,
Sri Vijay Vidyalaya College of Pharmacy,
Dharmapuri, Tamilnadu, India.

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I. INTRODUCTION:

(1)Flueggea leucopyrus is a more valuable medicinal plant. It is most commonly used types of medicinal plant. It is one of the species of flueaggea . Flueggea leucopyrus Willd is found throughout Sri Lanka's dry zones. In Ayurveda, Katupila (Flueggea leucopyrus) is also known as Heen Katupila and Sharapunkha (Flueggea leucopyrus). This plant can be growing up to 150 meters in many parts of South India. Its leaves are round and green in colour. The flowers resemble peppercorns in size and have a pale green colour. The tiny seeds resemble mustard seeds. Flueggea leucopyrus is a remedy for a wide range of illnesses such as cancer, liver hypertrophy, portal hypertension, abdominal, treat tooth ache, etc. The plant is sweet, cooling and tonic, useful for seminal weakness, burning sensations, and general debility and also tooth ache is treated with the bark of the stem (2) In several African and Asian nations, different species of the genus Flueggea are used to cure a wide range of illnesses, such as poliomyelitis, aplastic anemia, intestinal worms, edema, heavy menstruation, poliomyelitis, epilepsy, and malaria. The fruits of F. leucopyrus are eaten in India and Africa, while the leaves are used in Sri Lankan villages as a salad or "porridge." Since its therapeutic efficacy was discovered, F. leucopyrus has gained a lot of popularity among Sri Lankans. (3) Indians utilize the paste made from the leaves, which have disinfecting properties, to remove any unnecessary substances from bodily tissues without the need for surgery. For stomachaches, leaves are cooked and consumed twice daily. Additionally, the leaves are utilized to treat fibroids and piles.

Fig.01 flueggea leucopyrus

Taxonomical Classification

Taxonomy : flueggea leucopyrus
Kingdom : Plantae
Phylum : Tracheophyta
Class : Equisetosida C.gardh
Subclass : Malgiphiales
Order : Malpighighiales Juss.ex Bercht & J.presl
Family : Phyllanthaceae
Genus : Flueggea
Species : Leucopyrus

II. MATERIAL AND METHOD:

Leaves materials:

The flueggea leucopyrus leaves used in this study is collected from narayanapuram village, Dharmapuri. The collected sample was cleaned and washed thoroughly two to three times with running water and with distilled water. Then the collected leaves are shaded dried for 7 day, the moisture content removed by this drying process. Then the dried leaves are coarsely grinded and make a coarse power for the extraction process.

Materials:

- Soxhlet extractor
- Extraction thimble
- Solvents (Ethanol)
- Sample (coarse powder)
- Heating mantle
- Condenser.

PREPARATION OF EXTRACTS: Preparation of the extracts of the powdered leaves of flueggea leucopyrus is done by using ethanol as a solvent.
Ethanolic extract: The shade dried coarse powder of leaves 85gm was packed well in Soxhlet apparatus and subjected to continuous hot extraction with 450ml of ethanol for 18hrs. The extract was distilled in vaccum under pressure in order to remove the solvent completely. It was dried and kept in dedicator till experimentation obtained extract was weighed and percentage yield was calculated in terms of air dried powdered crude drug.

III. PHYTOCHEMICAL ANALYSIS OF FLUEGGEA LEUCOPYRUS LEAVES EXTRACTS

SCREENING OF ALKALOIDS:
(Mayer's reagent tests): The identification of alkaloids was carried out using the Mayer's test. A portion of the plant extract was mixed with 5ml of sulphuric acid in 50% ethanol. 1ml of Mayer's reagent was added drop by drop. The formation of a greenish color or cream precipitate indicated the presence of alkaloids**.**
SCREENING OF FLAVONOIDS:
(Sodium hydroxide test) 5ml of plant extract was mixed with few magnesium chips and 2 drops of concentrated hydrochloric acid were added and warmed. The presence of a pink/red color indicated the presence of flavonoids.
SCREENING OF REDUCING SUGAR: 1ml of the extract was added with 2ml of Fehling's reagent and 3ml of water. It was then boiled for 2minutes.
SCREENING OF SAPONIN:
(Frothing test) 3ml of the plant extract was added to 10ml distilled water and shaken vigorously for 30 seconds. Froth formation indicates the presence of saponins
SCREENING OF CARBOHYDRATE:
(Molisch test) Filtrate was treated with 1 drop of Molisch reagent and add 2ml of con.HCl was added from the side of test tube. The test tube was observed for formation of violet ring at the junction of two solutions indicated that presence of carbohydrates.
(Fehling test) Equal volume of Fehling's reagent A and B mixed together and 2ml of Mixture was added to plant extracts followed gentle heat, the mixture turned Brick red color.
SCREENING OF GLYCOSIDES: 2ml of chloroform, 2 ml of acetic acid were added to plant extract and allowed to cool, followed by addition of 2ml of concentrated H_2SO_4 changes the violet to blue then green, indicates the presence of steroidal nucleus that is glycone portion of glycoside.

SCREENING OF TANNINS:

(Bromine Water test) 5ml of plant extract was extracted with 20ml of 50% alcohol and then filtered. A few drops of bromine water were added to the resulting filtrate. The formation of a buff/white precipitate indicated the presence of tannins.

SCREENING OF STEROIDS:

(Libermann-Burchard test) 2ml of the test plant extract were mixed with 2 drops of chloroform and 2ml of acetic anhydride, along with 1ml of concentrated sulphuric acid added down the side of the tube. The formation of a reddish ring at the contact zone of the two liquids and a greenish color in the separate layer indicates the presence of steroids.

SCREENING OF TERPENOIDS: 5ml of extract was taken in a test tube and 2ml of chloroform was added to it followed by the addition of 3ml of concentrated sulphuric acid. Formation of reddish brown layer at the junction of two solutions confirms the presence of terpenoids.

Table.1. Results of phytochemical constituents

PHTOCHEMICAL TEST	WATER	PETROLIUM ETHER	CHLOROFORM	ETHANOL
Alkaloids	+	_	_	+
Glycosides	+	+	+	+
Saponin	_	+	+	_
Steroids	+	+	+	+
Flavonoids	_	_	_	_
Terpenoids	+	_	+	+
Tannin	+	_	_	+
Carbohydrate	_	+	_	_

- \+ Symbol indicates the presence of phytochemicals.
- \- Symbol indicates the presence of phytochemicals.

III. IN-VITRO ANTI BACTERIAL ACTIVITY OF FLUEGGEA LEUCOPYRUS

PREPARATION OF THE BACTERIAL INOCULUM:

Stock cultures were maintained at 4° C on slopes of nutrient agar and potato dextrose agar. Active culture for experiments were prepared by transferring a loop full of cells from stock cultures to test tubes of 50ml nutrient broth bacterial cultures were incubated with agitation for 24hours and at 37°c on shaking incubator and fungal cultures were incubated at 27°c for 3-5 days. Each suspension of test organism was subsequently stroke out on nutrient agar media and potato dextrose agar. Bacterial cultures then incubated at 37°c for 24 hours and fungal incubated at 27°c for 3-5 days. A single colony was transferred to nutrient agar media slants were incubated at 37°c for 24 hours and potato dextrose slant were incubated at 27°c for 3-5 days. These stock cultures were kept at 4°c. For use in experiments, a loop of each test organism was transferred into 50ml nutrient broth and incubated separately at 37°c for 18-20 hours for bacterial culture.

Well Diffusion method

The antibacterial activity and antifungal activity of crude extracts was determined by Well Diffusion method (Bauer *et al.*, 1996). The 2-20 µl of Nanoparticle extract was poured into the wells. After that, the plates were incubated at 37°C for 24 hours. Assay was carried into triplicates and control plates were also maintained. Zone of inhibition was measured from the edge of the well to the zone in mm. The tested cell suspension was spread on muller hintonagar plate and potato dextrose agar. well were put into the agar medium using sterile forceps. plant extract were poured on to wells. Then plates were incubated at 37°c for about 24 hours and control was also maintained. Zone of inhibition was measured from the clear zone in mm.

Antibacterial activity was performed by agar diffusion method (Van der Watt *et al.*, 2001). The stock culture of bacteria *(E.coli and Streptococcus)* were received by inoculating in nutrient broth media and grown at 37 % for 18 hours. The agar plates of the above media were prepared. Each plates was inoculated with 18 hours old cultures the bacteria were swab in the sterile plates. Cut the 5 wells Pour the extract in ratio 25 µl, 50 µl 75 µl 100 µl. All the plates were incubated at 37ºC for 24 hours and the diameter of inhibition zone was noted in cm.

Agar well diffusion method has been used to determine the antimicrobial activities and minimum inhibitory concentrations or plant extracts against Gram positive, Gram negative bacteria. The extracts exhibited antibacterial activities against tested microorganisms.

Solvent extract:

Table.2 Anti-bacterial activity of ethanolic extract with different concentration

Organisms Concentration	***E.Coli***	***Staphylococcus aureus***	***klebsiella pneumoniae***	***Candida albicans***
25 µl	4 mm	4 mm	5 mm	3 mm
50 µl	5 mm	5 mm	6 mm	4 mm
75 µl	7 mm	6 mm	7 mm	6 mm
100 µl	9 mm	7 mm	9 mm	8 mm
Standard	10 mm	10 mm	10 mm	10 mm

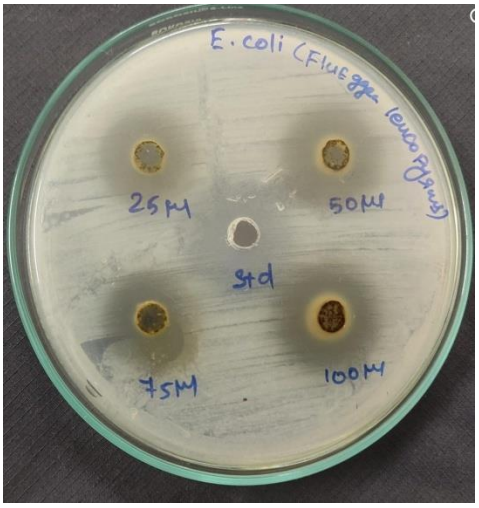


Fig .o2. *E.Coli*

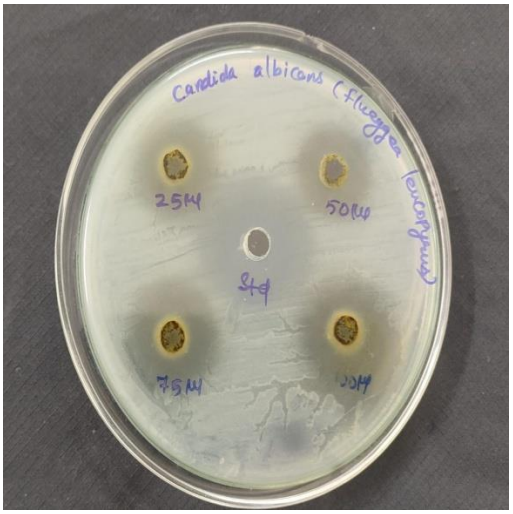


Fig.03. *Candida albicans*

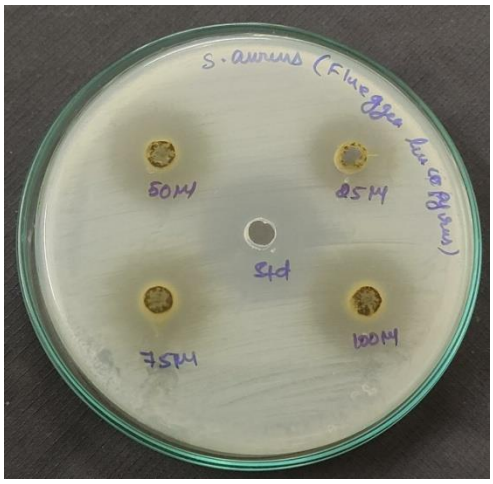
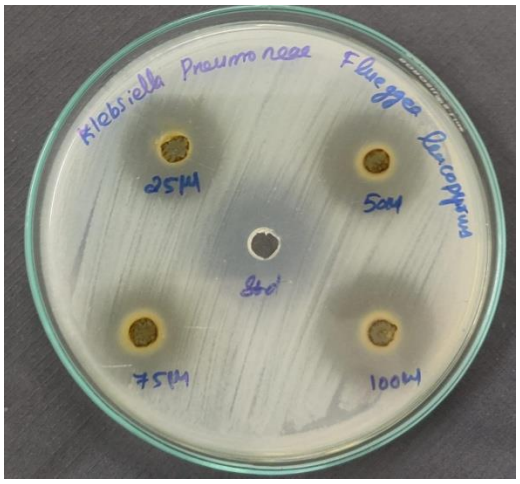


Fig.04. *Staphylococcus aureus*



F*ig.05. klebsiella pneumoniae*

IV CONCLUSION:

The give sample shows the Antibacterial activity against the Pathogenic Microorganisms like *E.Coli* and *Staphylococcus aureus* and *klebsiella pneumonia* and *fungus candida albicans.* The Solvent extract shows Good Antibacterial activity depending upon the size of the size of the zone.

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