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A Study of Antibacterial Activity of Chloroform Extraction of Cocculus Hirsutus

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ABSTRACT:

Antimicrobial activity has been demonstrated in vitriol for several herb and spice extracts .In this study Cocculus hirsutus a herb is used to identify the antibacterial activity using two organisms Staphylococcus aureus-Gram positive, Pseudomonas aeruginosa-Gram negative and streptomycin is used as a standard drug . Due to the presence of some alkaloids the antibacterial activity has been identified.The minimum concentration of 2000µg gives the maximum concentration as we compaired to standard. The Phytochemical and physical characters has been identified.The Soxhlet extraction with chloroform solvent is used and the resultant obtain liquid is used for antibacterial activity .The leaves which contain trichomes and paracytic stomata. This leaves is used for various diseases.

KEY WORDS: Cocculus hirsutus, alkaloids, trichomes, Phytochemical screening.

I. INTRODUCTION

The term "**MEDICINAL PLANT"** include various types of plants used in herbalism ("herbology" or "herbal medicine"). It is the use of plants for medicinal purposes, and the study of such uses. Plants have been used for medicinal purposes long before prehistoric period. Evidence exist that Unani Hakims, Indian Vaids and European and Mediterranean cultures were using herbs for over 4000 years as medicine. Indigenous cultures such as Rome, Egypt, Iran, Africa and America used herbs in their healing rituals, while other developed traditional medical systems such as Unani, Ayurvedic and Chinese Medicine in which herbal therapies were used systematically. Recently, WHO (World Health Organization) estimated that 80 percent of people worldwide rely on herbal medicines for some aspect of their primary health care needs. According to WHO, around 21,000 plant species have the potential for being used as medicinal plants. Treatment with medicinal plants is considered very safe as there is no or minimal side effects. These remedies are in sync with nature, which is the biggest advantage. Medicinal plants are considered as rich resources of ingredients which can be used in drug development pharmacopoeial, non- pharmacopoeial or synthetic drugs. A part from that, these plants play a critical role in the development of human cultures around the whole world. Over the past two decades, there has been a tremendous increase in the use of herbal medicine; however, there is still a significant lack of research data in this field. Therefore since 1999, WHO has published three volumes of the WHO monographs on selected medicinal plants

Introduction on Cocculus hirsutus.

Cocculus hirsutus is a tropical, invasive creeper with the common name broom creeper or Patalgarudi (Sanskrit). It is native to India, Pakistan, and tropical Africa. It is a vine climbing up to 3 metres (9.8 ft), with white to yellowish flowers and dark purple fruits 4 to 8 mm in diameter Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost. Therefore investigation on some active principles from traditional medicinal plants has become more important. The world health organization has also recommended the evaluation of the effectiveness of plants in condition where we lack safe modern drugs. Cocculus hirsutus, known well as Broom creeper, is found in moderately cool and hot regions of India particularly Tamilnadu, Bihar and Punjab. Cocculus hirsutus Linn (Menispermeaceae) is commonly known as Jal-Jammi. In Tamil this plant is known as Kattukkodi. Indian tribes use various plant parts of this plant for a wide range of ailments including constipation, kidney problems. In Tamilnadu Kaani tribes of Karaiyar using this plant for the treatment of skin disease, sexual debility and wound healing. It belongs to the family Meninspermaceae. In traditional medicine, the roots are used for the treatment of rheumatism, tuberculosis, leprosy, skin diseases, dyspepsia,

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pruritis, and flatulence, laxative, aphrodisiac, antipyretic and leaves are used in biliousness, eczema, gonorrhea, opthalmia, sexual debility and neuralgia. The juice of leaves coagulates in water and forms mucilage, which is used externally as a cooling and soothing agent in eczema and impetigo.

The leaf juice of this plant is used in the treatment of eczema. The roots of Cocculus hirsutus have been mentioned as bitter, acrid, laxative, tonic and diuretic. The plant has been reported to contain essential oil, β-sitosterol, and ginnol. The anti-inflammatory and analgesic activities of the roots were also reported .Since there is no report on the antimicrobial, Phytochemical and wound healing potential of this plant, an attempt was made to provide much information for the same.

Microorganism

Micro organisms are living organisms of microscopic size. Microorganism are closely associated with the health and welfare of human beings. Most microorganisms are unicellular. Unicellular organisms life process are performed by a single cell. Microorganisms can cause disease, spoil food and deteriorate materials. These microorganisms can be classified as Saprophyte, Parasites .Saprophytes live on dead or decaying organic matter parasites live on other forms of life.

BACTERIAL ORGANISMS USED FOR ANTIBACTERIAL ACTIVITY

1. Staphylococcus aureus

Staphylococcus aureus are gram positive cocci that occur in grape like clusters in golden yellow colonies. Colour production due to carotinoid pigments. They are spherical organism arranged in cluster. They grow readily on ordinary media with in a temperature 10^0C-40^0C at a pH 7.4-7.6. The colonies are circular convex, smooth which stain and produce golden yellow pigment. Pigment production enhanced when 1% glycerol mono acetate or milk is incorporated in the liquid medium produce uniform turbidity.

Pathogenicity

Localized, Contagious and metastatic lesions, Scaled skin syndrome , Staphylococcal food poisoning ,Other infection

Sensitivity to Antibiotics

Benzyl penicillin, cloxacillin, Cephalosporin, tetracycline, chloramphnicol, erythromycin, clindamycin, rancomycin, streptomycin, Gentamycin.

2. Pseudomonas aeruginosa

Pseudomonas aeruginosa are gram negative bacillus non-sporing, aerobic. They are straight or slightly curved rods. They are motile by polar, flagella. It is first named by Schroeter (1872) and Gersard (1882). It produces two pigments pyocyanin (bluish green) and fluorescent (greenish yellow). Pseudomonas aeruginosa found in small number of stools, from normal and it is a common contaminant of the hospital environment by water used.

Morphology

1.511m x 0.511m non-sporing, non-capsulated, gram negative bacillus, motility is due to one or more polar flagella, strains are fimbriate.

Pathogenicity

They produce respiratory tract infection systicfibrosin, urinary infection in burns lumbar puncture, pulmonary infection, ecthymiagangremosum, chronic non-neoplastic disorders and pneumonia. Medical equipment and product also contaminated.

Chemotherapy

Strains are regularly sensitive are Gentamycin, Carbenicillin, Polymyxin, Tobramycin, Active and passive vaccination is at present under investigation .Pseudomonas aeruginosa resistant to cetrimide and chloro.xylenol.

II. MATERIALS AND METHODS:

PLANT COLLECTION:

Cocculus hirsutus was collected from **THIRUPATTUR** and **JOLARPET** during the month of **OCTOBER 2022,** which is located in **THIRUPATTUR district**, Tamilnadu. This plant was then identified by **INDEGINIOUS PEOPLE**. The collected leaves of this plant were brought about to the laboratory, where the leaves were shade dried well for a 2 week.

Taxonomic classification of COCCULUS HIRSUTUS (Linn) Diels[1]

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Ranunculates
Family : Menispermaceae
Genus : Cocculus
Species : hirsutus

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Vernacular names[1]

Eng : Broom creeper, ink berry
Hin : Patalagarudi, Jaljamini
Kan : Sogadibali, Quesaribali
Mar : Patalagaruda kkot
San : Patalagaredah, chilihindah
Tam : Kattukkoti
Tel : Dusaratiga

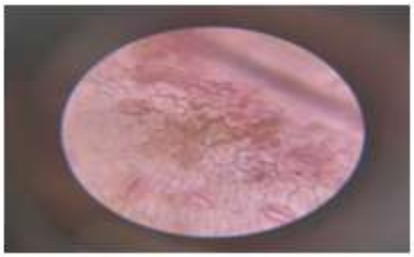
BOTANICAL DESCRIPTION

Hirsute climbers or stragglers. Leaves 2.5-5×2-2.5 cm, simple, ovate, obtuse, mucronate, truncate at base, softly villous. Flowers small, greenish-yellow. Male flowers in 5 to 6 cm long axillary cymose panicles. Calyx and corolla lobes 6, obovate. Stamens 6, enclosed by corolla lobes. Female flowers in axillary fascicles, rarely in racemes. Fruits globose, green, shining Pla te. Distributed throughout Africa and India in most districts.

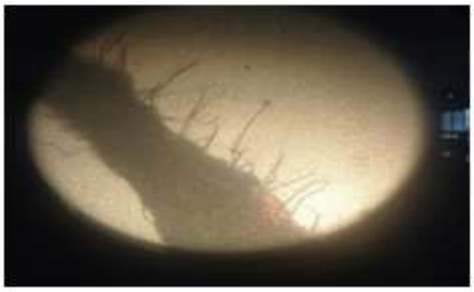
MACRO- AND MICROSCOPICAL CHARACTERS OF LEAVES

Leaves dorsiventral, variable, simple, ovate to ovate-oblong or slightly lanceolate with truncate to cordate base, apex sometimes mucronate, margins entire or slightly wavy, lamina hairy, greyish-white tomentose beneath. Veination reticulate with 5 to 6 pairs of alternating lateral veins; first two pairs of lateral veins arise basally giving a multicostate appearance; secondary and tertiary veinlets anastomose to form reticulation with free end included in meshes. Petiole greyish-tomentose, with a distinct swelling at proximal and distal ends. Leaves greenish, odourless and mucilaginous when fresh, brittle and powdery on drying, without characteristic taste, prolonged contact produces itching sensation Hairs or trichomes emerge from some epidermal cells. Hypodermis is made up of collenchymatous cells. Cortex consists of hexagonal cells, demarcated into inner region of larger cells and outer region of smaller cells,cells contain abundant chloroplast and sparsely distributed starch grains. Endodermis is single layered. Transverse section of leaf consists of lamina and midrib regions. Lamina exhibits upper and lower epidermis; lower epidermal cells smaller, measure , upper epidermal cells measuring epidermal cells rectangular, filled with chloroplast. Stomata anomocytic, found on lower epidermis, many, sunken, each surrounded by 4 to 6 epidermal cells. Mesophyll comprises of palisade and spongy parenchyma, cells filled with chloroplast and starch grains. Palisade cells columnar, 1-layered, except near midrib where it is 2 to 3-layered. Spongy parenchyma 2 to 3-layered, cells elongated, thin walled and enclose air spaces in between and excretory sacs. Midrib exhibits crescent shaped vascular bundle enclosed by sclerenchymatous bundle sheath. Next to bundle sheath lies parenchymatous ground tissue; some peripheral cells are collenchymatous; vascular bundle consists of xylem and phloem. Macerate of leaf exhibit epidermal cells measuring 6-8-9µ, with trichomes base and sunken anomocytic stomata ; unicellular trichomes which are long and ribbon shaped, measuring , filled with oil content in lower one third portion ; thin walled parenchyma cells .

Leaves of Cocculus hirsutus



Stomata of Cocculus hirsutus leave



Trichomes of Cocculus hirsutus leaves

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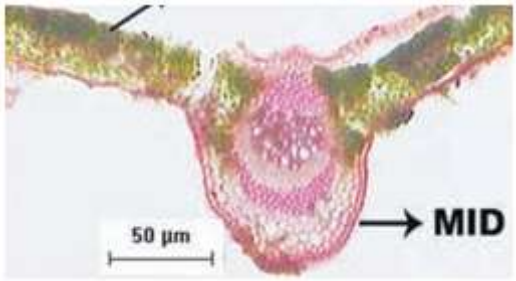

T S OF LEAVE
Figs1: Macro and microscopical characters of the leaves of Cocculus hirsutus

EXTRACTION:

It is a separation process involves the separation of medicinally active portions of plant or animal tissues from the Extraction inactive or inert components by using selective solvents in standard extraction procedures. In this method the wanted components are dissolved by the use of selective solvents known as menstrum & undissolved part is marc. After the extraction unwanted matter is removed. Extracts are prepared by using chloroform or other

Suitable solvent. Extract: extracts can be defined as preparations of crude drug METHODS OF EXTRACTION

EXTRACTION METHODS	
CONVENTIONAL METHODS	NON-CONVENTIONAL METHODS
a) Maceration b) Infusion c) Digestion d) Percolation e) Decoction f) Soxhlet extraction g) Serial exhaustive extraction	a) Ultrasound- assisted extraction b) Supercritical fluid extraction c) Pressurized liquid extraction d) Microwave- assisted extraction

Powder of Cocculus hirsutus leaves

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Soxhlet extraction of Cocculus hirsutus

Extraction of Cocculus hirsutus Using Chloroform as solvent

In this study, Soxhlet technique is used for chloroform extraction of Cocculus hirsutus**.**

SOXHLET EXTRACTION

Leaf was dried in the laboratory at room temperature for 7 days. All dried samples were ground well into a fine powder in a mixer grinder. The powder was extracted by Soxhlet extraction method using chloroform as solvent. Then 25 mg of each crude plant extract was dissolved in chloroform solvent (1,000 µl) of the solvent to give a final concentration of crude extract in solvent of 50 mg/ml. Then, this extract was used for antibacterial activity

PHYTOCHEMICAL SCREENING

Preliminary analysis of extracts was carried out to identify the presence of various phytoconstituents by employing standard protocols .The results were summarized in Table after conducting the following chemical tests.

1. Tests for Alkaloids:

(a) Dragendorff"s test: By adding 1 ml of Dragendorff's reagent to 2 ml of extract, an orange red precipitate was formed, indicating the presence of alkaloids.

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2. Tests for Flavonoids:
(a) Alkaline reagent test (flavonones): Two to three drops of sodium hydroxide were added to 2 ml of extract. Initially, a deep yellow colour appeared but it gradually became colourless by adding few drops of dilute HCL, indicating that flavonoids were present.

3. Test for Flavones:
To the extract, 10% Sodium hydroxides was added. Yellow to orange colour showed presence of Flavones

4. Test for Phenolic compounds and Tannins:
Ferric chloride test: 2ml of 5% neutral ferric chloride solution were added to 1 ml of extract, the dark blue colouring indicating the presence of phenolic compounds and tannins.

5. Test for Carbohydrates:
(a) Molish test: Few drops of alcoholic a-naphthol solution were added to 2 ml of extract. Later, few drops of concentrated H2SO4 were added along the walls of test tube. At the junction of two liquids, a violet colour ring appeared, indicating that carbohydrates were present.
(b) Benedict's test: To 5 ml of Benedict's reagent, 8-10 drops extract were added, then heated for five minutes; the resulting dark red precipitate indicated the presence of carbohydrates.
(c) Reducing sugars (Fehling's test): To 2 ml of extract, an equal volume of Fehling's (A & B) solution was added and heated for five minutes, the resulting red/dark red precipitate Indicating presence of carbohydrates.

6. Tests for Saponins:
A drop of Na2CO3 solution was added to 5 ml of extract in a test tube. After vigorous shaking, it was left to rest for five minutes. Foam formation indicated the presence of saponins.[6]

7. Test for Tannins:
To the extract, little amount of lead acetate solution was added. White precipitate indicates presence of tannins.

8. Test for Amino acids:
To the extract added Ninhydrin reagent and warmed it. Violet or Pink colour indicates the Presence of amino acids.

9. Test for Quinones:
To the little amount of extract sodium hydroxide was added, red or blue green colour indicates the presence of Quinones.

10. Test for glycosides:
(a) Keller killani test: A solution of 0.5ml, containing glacial acetic acid and 2-3 drops of ferric chloride, was mixed with 2ml of extract . later,1ml of concentration H2SO4, was added along the walls of the test tube .The appearance of deep blue colour at the junction of two liquid indication the present of cardiac glycoside

PHYSICAL EVALUTION:
Physico-chemical analysis involves a ash value such as total ash (%) acid-insoluble ash(%) , sulphate ash, water soluble ash and loss on drying.

DETERMINATION ASH VALUE:
a) Total Ash value:
2 gm of powder was weighed accurately in a previously ignited and tarred silica crucible. The material was then ignited by gradually increasing the heat to 500-600°c until it appear white indicating absence of carbon. It is then cooled in a desicator and total ash in mg per gram of air dried material is calculated.

b) Acid Insoluble Ash Value:
To the crucible containing total ash, 25 ml of HCl was added and boiled gently for 5 minutes, and then about 5 ml of hot water was added and transferred into crucible. The insoluble matter was collected on an ashless filter paper. This was then washed with hot water until filtrate is neutral and the filter paper along with the insoluble matter was transferred into crucible and ignited to constant weight. The residue was then allowed to cool and then weighed.

c) Sulphated ash:
Take 1-2gm of powder in an accurately weighed crucible; ignite gently at first until the substance thoroughly charred. Cooled, moistened the residue with 1ml of sulphuric acid, heat gently until white fumes are no longer evolved & ignite at 800°c + 25°c until black particles have disappeared. Allow the crucible to cool and weigh. Repeat the operation two successive weighing in don't differ by more than 0.5mg.

d) determination of loss on drying:
Loss on drying was determined by weighing about 2gm of the powder material in

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previously weighed dried Petridis (tarred evaporating dish) and dried in an oven at 105-110°c, till two consecutive weights, which do not differ by more than 5mg. The weight after drying was noted and loss on drying was calculated. The percentage was expressed as %w/w with reference air dried sample.[5]

Name of the organisms used for the study

Staphylococcus aureus - Gram positive

Pseudomonas aeruginosa - Gram negative

Media used for growth of bacteria

Intended Use:

Recommended for testing susceptibility of common and rapidly growing bacteria using antimicrobial discs by the Bauer - Kirby method. Manufactured to contain low levels of thymine, thymidine, calcium and magnesium.

Composition of Muller Hinton broth:

Table:1

Ingredients	Grams /liter
Beef infusion	2.0
Starch	1.5
Casein hydrolysis	17.5

Final pH 7.4 +/- 0.2 at 25°C

Store prepared media below 8°C, protected from direct light. Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C.

Test organisms:

The bacterial spp. used for the test were Staphylococcus aureus (S. aureus), and Pseudomonas aeruginosa (P. aeruginosa). All the stock cultures were obtained from Micro lab.

Culture media and inoculums preparation:

Nutrient broth (Himedia, India.) were used as the media for the culturing of bacterial strains. Loops full of all the bacterial cultures were inoculated in the nutrient broth (NA) at 37°C for 72 hrs.

Directions:

Dissolve 21 g in 1 litre of distilled water. Sterilize by autoclaving at 121°C for 15 minutes.

Disc-diffusion method:

The disc diffusion method (DDM) is classified as an agar _diffusion method (ADM) because the plant extract to be tested diffuses from its reservoir through the agar medium seeded with the test microorganism. Generally, the reservoir is a filter paper disk, which is placed on top of an agar surface. If tested plant extracts or isolated compounds are microbiologically active, an inhibition zone develops around the filter paper disk after incubation. The diameter of the inhibition zone properly describes the antimicrobial potency of plant extracts or individual compounds. It should be mentioned that DDM is not an appropriate method for lipophilic extracts (eg, EOs) because the diffusion of the water-insoluble EO and its compounds from a filter paper disk into the agar medium is insufficient.

The antibacterial activity of the test samples were carried out by disc diffusion method. The target microorganisms were cultured in Mueller-Hinton broth and incubated for 24 h. The Petri dishes containing Mueller-Hinton agar medium were cultured with diluted bacterial strain. Test samples were loaded in sterile disc and placed on the culture medium. Then the inoculated plates were incubated at 37°C for 24 h. Streptomycin was used as a positive control. The diameter of the clear zone around the sample was measured and expressed in millimeters as its antibacterial activity.

The extracts obtained above were screened for their antibacterial activity in comparison with standard antibiotic streptomycin (10 µg/mL) in-vitro by disc diffusion method using S. aureus and P. aeruginosa as test organisms. Each extract was individually loaded on the 3 mm sterile disc at the concentration of 500 µg/mL, 1000 µg/ mL and 2000 µg/mL and subjected to antibacterial

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activity. The results were recorded by measuring the zone of growth inhibition surrounding the disc. The experiments were done in triplicate.

III. RESULTS

- After the successful physicochemical analysis study was done and it shows 5.7%w/w total ash content, 0.57%w/w of acid insoluble ash indicates the presence of siliceous matter in sample, 8.5%w/w sulphated ash and 7.92%w/w loss on drying. The results are shown in table1

Table 2: Physical Evolution of chloroform extraction of Cocculus hirsutus

S.No.	Parameters	Results (%w/w)
1.	Total ash	5.7
2.	Acid-insoluble ash	0.57
3.	Sulphated ash	8.5
4.	Loss on drying	7.92

- By using the powder of cocculus hirsutus, the preliminary phytochemical study was done .It shows the presence or the absence of the chemical constituent. The detailed results are shown in table 2

Table 2 :Chemical Evaluation of chloroform extracts of cocculus hirsutus

CHEMICAL TEST	PRESENCE OR ABSENCE OF CHLOROFORM EXTRACT OF COCCULUS HIRSUTUS
Alkaloids	+
Flavonoids	-
Test for Phenolic compounds & tannis	-
Test for carbohydrate	-
Teat for Saponins	+
Test for reducing sugars	+
Test for flavones	–
Test for tannins	–
Test for amino acids	–

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Test for quinines	–
Test for proteins	–
Test for glycoside	–
Formation of mucilage in water	+

Present-(+)
Absent-(-)

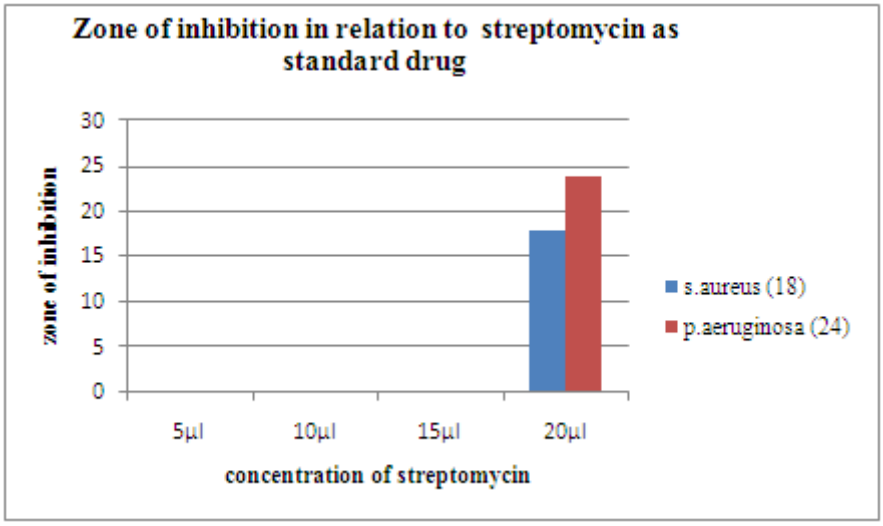
Antibacterial activity study report

Result:

METHODOLOGY OF GRAPH

The data obtained through the determination of (MIC) Minimum inhibitory concentration from the association of antibiotics with extracts observe anti-biotic effects. The results revealed that extracts have same MIC on both bacteria. The extract of cocculus hirsutus on P.aeruginosa and s. aureus bacteria show s both the zone of inhibition at 2000µg is (10) and control test used as the streptomycin at 20µl shows zone of inhibition of P.aeruginosa is (24) and S.aureus is (18) are shown in below as graphical representation .

Samples	**Zone of Inhibition (mm)**					
	Microorganisms					
	S.aureus			**P.aeruginosa**		
CT	500 µg	1000 µg	2000 µg	500 µg	1000 µg	2000 µg
	-	-	**10**	-	-	**10**
Streptomycin (20µl)	**18**			**24**		



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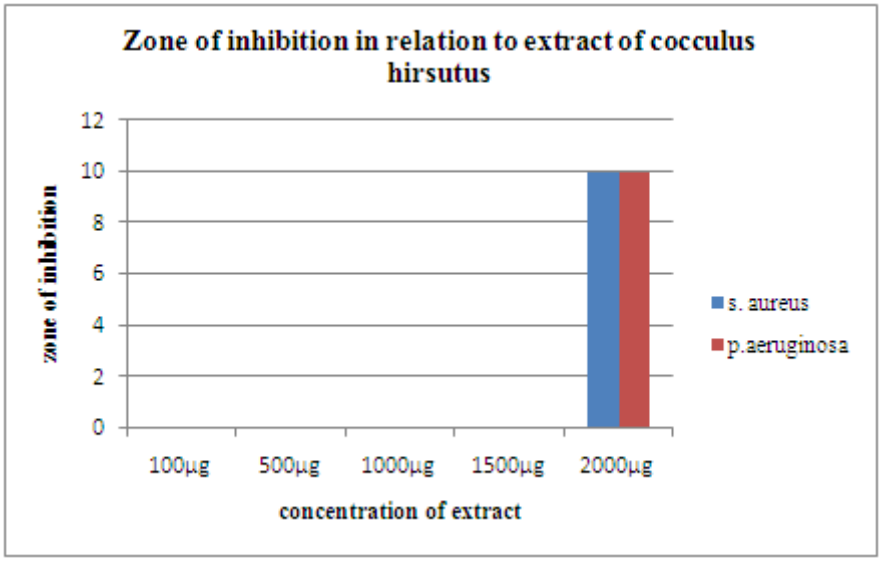
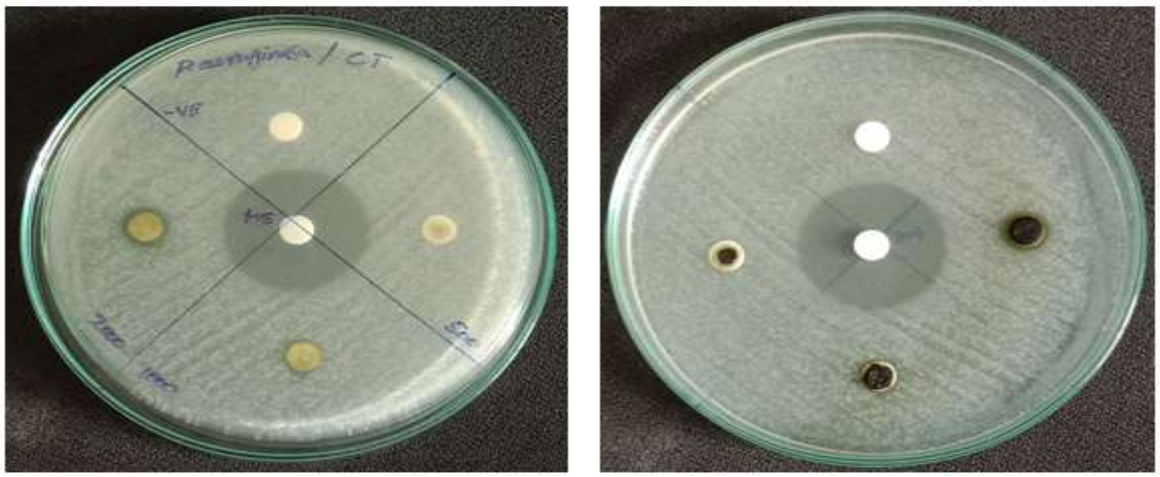
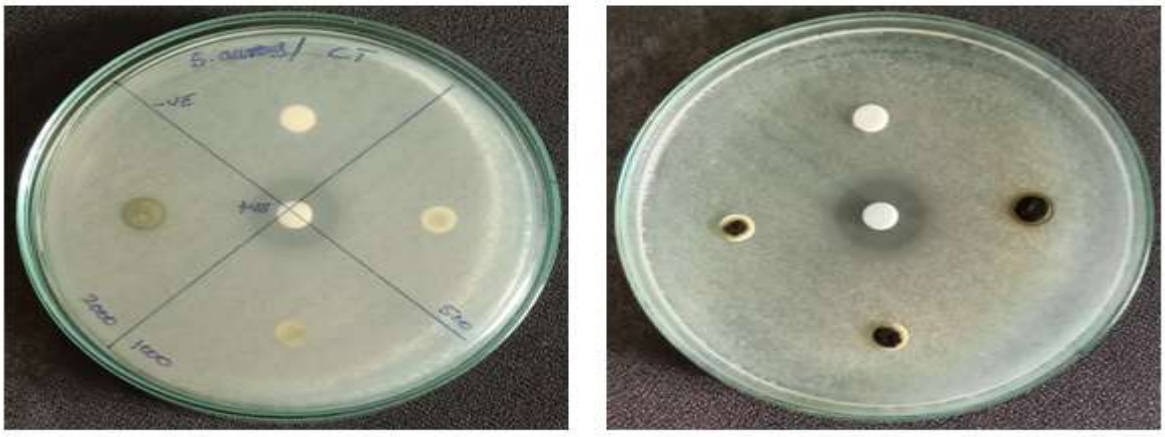




Antibacterial activity of chloroform extracts leaf of Cocculus hirsutus (Pseudomonas aeruginosa – gram negative)



Antibacterial activity of chloroform extract leaf of Cocculus hirsutus(Staphylococcus aureus – gram positive)

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IV. CONCLUSION

Based on the results, it can be concluded that the Cocculus hirsutus plant extracts have great potential as antibacterial components against microorganisms and they can be used in the treatment of infectious diseases caused by resistant microorganisms. Further work is needed to isolate the secondary metabolites from the extracts studied in order to test specific antibacterial activity and the underlying mechanisms.

Staphylococcus aureus and Pseudomonas aeruginosa gram positive and gram negative bacteria causes various infections in our body .By the test result both bacteria are resistant to 2000 µg of chloroform extract of cocculus hirsutus about 10mm zone of inhibition. Due the presence of alkaloid secondary metabolite in the cocculus hirsutus the antibiotic activity has been proved by the 2 microorganism gram+ve and gram–ve

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