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EVALUATION OF ANTIMICROBIAL ACTIVITY OF LEAVES AND STEMS OF COCCULUS HIRSUTUS

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1. ***Abstract*** :-This study evaluates the antimicrobial activity of ethanolic extracts from the leaves and stems of Cocculus hirsutus. The investigation targeted various test microorganisms, including Penicillium, Aspergillus niger , Pseudomonas aeruginosa and Candida albicans. The agar well diffusion method was employed to assess the efficacy of the extracts, with gentamycin serving as the standard reference drug. Results indicated that the ethanolic extract of Cocculus hirsutus exhibited significant antimicrobial properties. The effectiveness of the extract varied across the different microorganisms, suggesting a broad spectrum of activity. This study highlights the potential of Cocculus hirsutus as a source of natural antimicrobial agents, providing a basis for further research and development in medicinal applications.

2. INTRODUCTION:-

Herbal medicines, derived from plant, animal and mineral sources, have been used to treat human disease since antiquity. Today, it is estimated that 80% of people in developing countries still rely on traditional medicine, largely based on plant and animal species, as their primary health care. The demand for herbal medicines is growing day by day, as the use of herbal medicines is becoming increasingly popular due to the toxicity and side effects of traditional allopathic medicines, leading to a sudden surge in herbal drug manufacturing [1]. Herbal medicines have been used as the main remedy in traditional systems of medicine since antiquity, and continue to be used today because of its biomedical benefits and place in cultural beliefs across many parts of the world, and have made a great contribution to the maintenance of human health. Currently, 80% of the world population relies on plant-derived medicine for primary health care, due to its lack of side effects. Currently, 25% of pharmaceutical prescription drugs in the United States contain at least one component

derived from plants[2]. In the last hundred years, about 121 pharmaceutical products have been developed on the basis of traditional knowledge collected from a variety of sources. Plants should be studied to learn more about their characteristics, safety, and effectiveness[3].

The plant selected for this study is Menispermaceae Menispermaceae Cocculus Hirsutus L., a perennial climber found in both tropics and subtropics.Cocculus hirsutus is a diuretic, a laxative, a hypolipidic, and a spermatogenic plant[4]. In ancient times, this plant was famous for its ability to heal all types of wounds, cuts, and boils in a very short period of time and with very little pain. It is also used to treat gonorrhea, spermatozoa, urinary problems, diarrhoea, and hypoglycemia[5-6].

Antimicrobial resistance (AMR) poses a significant global health threat, undermining our ability to treat infectious diseases effectively. Here's a detailed background on AMR and the importance of exploring alternative antimicrobial agents[5-6]..

Antimicrobial Resistance (AMR):

• Antimicrobial resistance occurs when microorganisms (bacteria, viruses, fungi, parasites) evolve mechanisms to withstand the effects of antimicrobial drugs.

• The misuse and overuse of antimicrobial agents, including antibiotics, antivirals, antifungals, and antiparasitic drugs, accelerate the development of resistance.

• AMR leads to treatment failures, prolonged illnesses, increased healthcare costs, and higher mortality rates [7].

Global Impact of AMR:

• AMR affects individuals, communities, and healthcare systems worldwide.

• It compromises the treatment of common infections such as pneumonia, urinary tract infections, and sexually transmitted diseases, as well as life-threatening conditions like sepsis and tuberculosis.

• AMR increases the complexity of medical procedures, including surgeries, chemotherapy, and organ transplants, as patients are more susceptible to infections[8].

Economic Burden:

• The economic impact of AMR is substantial, resulting from increased healthcare expenditures, productivity losses, and agricultural implications.

• Treating drug-resistant infections often requires more expensive and less accessible second-line or last-resort antibiotics, further straining healthcare budgets.

• In agriculture, AMR affects livestock health, food safety, and agricultural productivity, impacting livelihoods and food security[9].

Need for Alternative Antimicrobial Agents:

• The rise of antimicrobial resistance underscores the urgent need to explore alternative antimicrobial agents to combat resistant infections.

• Traditional antibiotics are becoming less effective, necessitating the discovery and development of novel antimicrobial compounds. • Alternative antimicrobial agents offer potential solutions to address the gaps in current treatment options, providing alternatives for both human and veterinary medicine[10].

Herbal Extracts as Alternative Antimicrobial Agents:

• Herbal extracts represent a rich source of bioactive compounds with diverse antimicrobial properties.

• Plants produce secondary metabolites, such as alkaloids, flavonoids, terpenoids, and phenolics, which exhibit antimicrobial activities against a wide range of pathogens.

• Traditional medicine systems, including Ayurveda, Traditional Chinese Medicine, and Indigenous healing practices, have long utilized plant-based remedies for treating infections[11].

Advantages of Herbal Antimicrobials:

• Herbal antimicrobial agents offer several advantages, including broad-spectrum activity, potential synergy between multiple compounds, and reduced likelihood of inducing resistance compared to conventional antibiotics.

• Many herbal extracts possess additional pharmacological properties, such as anti-inflammatory, antioxidant, and immunomodulatory effects, which may enhance their therapeutic potential.

• Utilizing herbal antimicrobials aligns with principles of sustainable healthcare and promotes the conservation of biodiversity[12].

Research and Development Initiatives:

• There is growing interest in exploring the antimicrobial properties of herbal extracts through interdisciplinary research involving pharmacology, microbiology, ethnobotany, and biotechnology.

• Research efforts focus on identifying bioactive compounds, elucidating their mechanisms of action, optimizing extraction methods, and evaluating their efficacy and safety in preclinical and clinical studies.

• Collaboration between traditional knowledge holders, scientists, healthcare professionals, and policymakers is essential for advancing research and integrating herbal antimicrobials into mainstream healthcare practices[13].

2.1 Cocculus hirsutus (L.) Diels

Cocculus hirsutus, commonly known as **broom creeper** or **Patalgarudi** in Sanskrit, is a tropical flowering plant with the common name broom creeper or Patalgarudi[14].it is native to Asia , and Tropical Africa[15]. This fascinating vine can climb up to **3 meters (9.8 feet)** and exhibits the following characteristics:

- **Habit**: Cocculus hirsutus is a straggling scandent twiner. Its young parts are full of long weak hairs (villous), and its branches are marked with grooves or ridges.
- **Leaves**: The leaves are ovate-oblong, hastate, obtuse, or subacute with a sharp short point. They are subcordate at the base and covered with soft hairs on both sides. The petioles are densely hairy.[16,17,18]
- **Inflorescence**: Cocculus hirsutus has small axillary cymose panicles.
- **Male Flowers**: Male flowers appear first in small axillary cymose panicles. Their pedicels are slender, and the bracts are minute and hairy. Sepals are oblong-ovate, with the inner three being larger. Petals, numbering six, are shorter than the sepals, embracing the stamens and being thinly membranous. They also have auricles.[19,20,21]
- **Female Flowers**: Female flowers appear later in axillary clusters of 2-3 together. Their petals are larger than those of male flowers, thick, and fleshy.

Fig.no.1 : Cocculus Hirsutus

Family	MENISPERMACEAE (Moonseed Family)
Hindi Name	GILOY FAMILY (गिलोय परिवार)
Species Name	Cocculus hirsutus (L.) W.Theob.
Common Names	Broom Creeper, Ink Berry
Vernacular Names	Shindal Makki (Urdu), Dhagadphodi (Kannada), Vasamul, Vasanvel, Karsom, Jalmanni, Karmol, Dev-pala (Marathi)
Habitat	Dry deciduous forests, scrub jungle foothills
Habit	Woody roots; commonly found in fields. Roots used for chronic rheumatism, muscular diseases; leaves for eczema. Found up to 750m elevation.
Distribution	Widely distributed across various districts in Andhra Pradesh, Karnataka, Maharashtra, Tamil Nadu, and Odisha.

◆ **Traditional Uses**

Various publications have mentioned the traditional uses of C. hirsutus as practiced by the ethnic peopleinSouthAsia. TheKoyasusetheleafpaste,whichisappliedonheadforitscoolinge ect[23–27]. The juice of the plant mixed with sesame oil is applied on the head and body to reduce heat. To allay the stomach heat and for the treatment of blood dysentery, the plant-paste is applied over the navel region [26]. The leaves are used to treat prurigo, impetigo, eczema, sores, cuts, wounds and other skin disorders [28,29]. Leaves are also used in the treatment of urine disorders, fever, leucorrhoea and acute gonorrhea [27,30–32]. The leaves and stems are used in the treatment of conjunctivitis and other eye disorders [25,28,33]. The leaf powder is given orally for the treatment of dysentery and diarrhea [34]. The stem is used in the treatment of stomach disorders [26,28]. The roots are bitter, alterative and laxative and are used in the treatment of fever, skin irritation, rheumatism, gout, syphilitic cachexia and also in children for the treatment of stomach-ache [28,31,32,35–41]. The extract of stems and roots are used as a sedative, hypotensive, cardiotonic and spasmolytic [35]. The root is made into paste and mixed with water and given orally to reduce stomach pain [42]

◆ Chemical Constituents

- Although the chemical constituents of C. hirsutus are widely used in traditional medicine and its pharmacological effects have been well studied, its chemical constituents have not been sufficiently studied.Earlier studies in the 1960s and 1970s reported the presence of alkaloids through preliminary phytochemical screenings and the isolation and identification of some alkaloids such as trilobine, isotrilobine, cocalaurine and magnoflorine [43-45] and other compounds, namely methyleterntoster and methyleterntoster. Inositol [46]. Some studies conducted in the 1980s or later reported several alkaloids from different plant parts. List of alkaloids isolated from C. hirsutus are shown in Table 1 and their
- structure is shown in Figure . Three flavonoids, rutin, licritin
- and quercetin, have also been reported from the leaves [47]. The triterpene derivative hirsudiol was also reported from the ethanolic extract of the whole plant [48]. Similarly, -sitosterol and 28-acetylbotulinum were isolated from the aqueous extract of parts of the air [49]. Many studies have reported preliminary phytochemical screening of extracts and the presence of carbohydrates, steroids, alkaloids, glycosides, flavonoids, tannins and saponins [50].
- Further, Medicines2020,7,0069 4of10 gas chromatography -mass spectroscopy (GC-MS) analys is of the extracts have revealed the presence of various compounds[50,51].

S.N.	Compound Name	Plant Part/Extract	Reference
1	Jamtine	Whole plant/ethanol extract	[32]
2	Jamtine N-oxide	Stems and roots	[32,52]
3	Haiderine	Whole plant/ethanol extract	[32]
4	Hirsutine	Whole plant/ethanol extract	[32,53]
5	Cohirsitine	Whole plant/ethanol extract	[32]
6	Cohirsitinine	Whole plant/ethanol extract	[31]
7	Cohirsine	Whole plant/ethanol extract	[32,55]
8	Cohirsinine	Whole plant/ethanol extract	[32,56]
9	Corsutine	Stems and roots/ethanol extract	[57]
10	Coclaurine	Stems and roots, whole plant/ethanol extract	[32,43,44]
11	Shaheenine	Stems and roots	[22,43]
12	Magnoflorine	Stems and roots	[22,33]
13	Trilobine	Stems and roots	[32,43,44]

14	IsoTriloboline	Stems and roots	[32,42]
15	Cocsulene-N-2-oxide	Whole plant/ethanol extract	[32]

Table NO.1:Chemical Constituents

Structures of alkaloids reported from Structures of alkaloids reported from C. hirsutus.

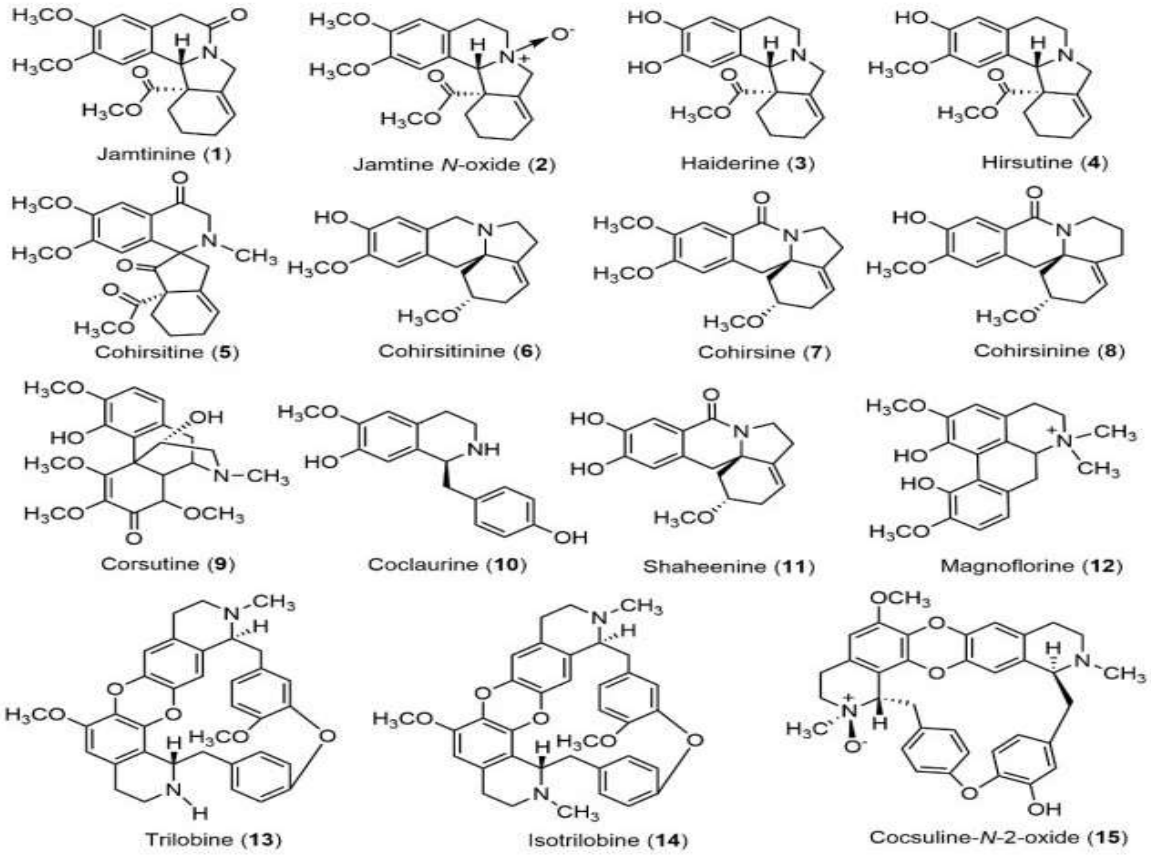

Fig.No.2 : Chemical structures

- **Pharmacological Activities**

Various pharmacological activities have been reported for the extracts and isolated compounds from the di erent plant parts of C. hirsutus.

- **Anti-Microbial Activity**

Jethva et al. carried out the anti-mycobacterial activity of the aqueous extract of C. hirsutus against Mycobacterium tuberculosis H37Rv and the extract showed potent anti-mycobacterial activity with the inhibition percentage of 80.26% [52]. Gupta et al. evaluated the anti-mycobacterial activity of ethanol extract of the leaves of C. hirsutus against M. tuberculosis H37Rv and various multidrug resistant (MDR) strains. The extract showed potent anti-mycobacterial activity against M. tuberculosis H37Rv and MDR strains JAl-19187, JAL-19126, JAL 19049, JAL 19111 and JAL- 19188 with MIC values of 500, 250, 500, 250, 500 and 500 g/mL, respectively [53]. Devi et al. carried out the anti-bacterial activity of methanol, ethanol and aqueous extract of the leaves of C. hirsutus (a concentration of 25, 50, 75, 100 L) using clinical bacterial isolates such as Escherichia coli, Salmonella typhi, Micrococcus luteus, Staphylococcus aureus, Acetobacter la , Proteus mirabilis and Bacillus cereus and the extracts showed potent antibacterial activities [54]. Nayak and Singhai carried out the anti-bacterial activity evaluation of the di erent extracts of roots of C. hirsutus against Staphlococcus aureus, Escherchia coli, Pseudomonas aeruginosa and Salmonella typhi and the ethanol extract showed potent antibacterial activity [55]. Devi et al. evaluated the anti-fungal activity of the aqueous extract of C. hirsutus against Rhizopus arrhizus, Sclerotium rolfsii and Fusarium solani fungal strains and the extract showed antifungal activity against S. rolfsii and F. solani [56]

- **Anti-Malarial and Insecticidal Activity**

Brahmam and Sunita carried out the in-vitro antimalarial activity of di erent extracts of roots of C. hirsutus against two Plasmodium falciparum strains, i.e., 3D7 (chloroquine sensitive strain) and K1 (chloroquine resistance strain). The chloroform and methanol extracts showed potent activity against both strains. [57]. Elango et al. evaluated the larvicidal activity of the leaves of C. hirsutus against malaria vector Anopheles subpictus larvae and the di erent extracts showed potent activity with a percentage mortality at 24 h and emergence inhibition values: hexane extract (60 2.04 and 75 2.44), choloroform extract (78 2.56 and 85 1.50), ethyl acetate extract (86 1.29 and 69 1.71), acetone extract (100 0.00 and 68 2.13) and methanol extract (81 1.08 and 100 0.00) [58]. Elango et al. also reported the larvicidal activity of the ethyl acetate and acetone extracts of the leaves of C. hirsutus against Culex tritaeniorhynchus and Anopheles subpictus [59]

- **Anti-Cancer Activity**

De Wet et al. carried out the anti-cancer activity of crude alkaloidal extract of rhizomes of C. hirsutus in three cancer cell lines, i.e., breast (MCF7), melanoma (UACC62) and renal (TK10) cell lines and the extract showed moderate anticancer activity [60]. Thavamani et al. carried out the in-vitro cytotoxic activity of the

methanolic extract of C. hirsutus against HeLa cell line and the results showed an IC50 value of 111 g/mL [61]. Another study evaluated the anti-cancer activity of the plant C. hirsutus against Dalton's lymphoma ascites (DLA) cells in mice. The methanolic extract of C. hirsutus showed significant cytotoxic activity with an IC50 value of 84.56 mg/mL in MCF-7 cancer cell line in-vitro. The extract also showed in-vivo antitumor activity as the doses of 200 and 400 mg/kg body weight significantly reduced the packed cell volume, tumor cell count, and restored the hematological and serum biochemical parameters towards the normal values [62]. Medicines 2020, 7, 0069 6 of 10

- **Immunomodulatory Activity**

Mallik and Nayak evaluated the immunomodulatory activity of the combination (1:1, 2:1 and 1:2 ration) of leaves of C. hirsutus and flowers of Sesbania grandiflora (L.) Pers. (Fabaceae) in mice. The 1:1 combination mixture showed potent immunostimulatory activity [63]. Rastogi et al. evaluated the immunostimulatory activity of aqueous and ethanolic extract of aerial parts of C. hirsutus in normal as well as cyclophosphamide induced immunosuppressed rats. The extracts showed an dose dependent increase in the carbon clearance, humoral antibody (HA) titre, delayed type hypersensitivity (DTH) and white blood cell (WBC) count in a dose dependent manner and authors concluded that the extract was e ective to stimulate the immune system and also to protect from the immunosuppressant [64].

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- **Anti-Oxidant Activity**

Srikanta and Dharmesh evaluated the anti-oxidant activity of the aqueous extract of the leaves of C. hirsutus using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical and reducing power assay, and the extract showed potent DPPH free radical scavenging assay with IC50 values of 2.75 0.3 g gallic acid equivalents (GAE)/ml and reducing power activity with the value of 65.17 4.8 U/mg GAE. The extract also showed the total phenolic content of 31.83 3.1 mg GAE/g [67]. Rakkimuthu et al. studied the anti-oxidant activity of C. hirsutus. The results showed potent DPPH free radical scavenging activity, ABTSfree radical scavenging activity, nitric oxide scavenging activity, reducing power, inhibition of lipid peroxidation and metal chelating activity assay as ascorbic acid [68].

- **Hepatoprotective Activity**

Thakare et al. evaluated the hepatoprotective activity of the methanolic extract of C. hirsutus in albino Wister rats with ethanol-induced hepatotoxicity and the oral administration of the extract at doses of 100, 200 and 400 mg/kg significantly lowered the levels of AST, ALT, ALP, LDH, direct and total bilirubin and cholesterol [69].

- **Diruetic and Nephroprotective Activity**

Ganapaty et al. studied the diuretic activity of the aqueous extract of the aerial parts of C. hirsutus in normap mice and the extract at a dose of 100 and 200 mg/kg, p.o. showed significant increase in the urinary concentrations of Na+ and K+ suggesting potent diuretic activity [70]. Badole et al. carried out the acute and chronic diuretic activity of the ethanolic extract of leaves of C. hirsutus in normal rats and the extract significantly increased the urinary concentrations of Na+ and K+ [71]. Gadapuram et al. reported the potent nephroprotective activity of the ethanolic extract of the leaves of C. hirsutus in 5/6 nephrectomized rat model [72].

➢ **Other Activities**

Ranjan et al. evaluated the wound healing activity from the leaves of C. hirsutus and reported that the methanolic extract showed the highest wound healing activity among the tested groups whencompared to the standard [73]. Ganapaty et al. evaluated the laxative activity of the aqueous extract of the aerial parts and the extract showed significant laxative activity at the doses of 100 and Medicines 2020, 7, 0069 7 of 10 200 mg/kg, p.o. [70]. Sangameswaran and Jayakar evaluated the spermatogenic activity C. hirsutus in streptozotocin-induced diabetic rats and the increase in the sperm count was observed at a concentration of 400 mg/kg p.o (102.83 1.85) and 800 mg/kg p.o. (117.83 3.49) when compared to the normal group (74.83 1.97) [69]. Elango and Rahuman reported the potent anti-parasitic activity of extracts of leaves of C. hirsutus.

2.2 Test Organisms

1. Penicillium

Fig.no.3 : *Penicillium*

I. **General Information:**

- Penicillium is a genus of filamentous fungi belonging to the phylum Ascomycota.
- It is widely distributed in various terrestrial and aquatic environments, including soil, decaying organic matter, air, and water.
- Penicillium species play significant roles in ecosystems as decomposers, contributing to the breakdown of organic materials.

II. Taxonomy and Classification:

- Penicillium is classified within the family Trichocomaceae and the order Eurotiales.
- The genus includes over 350 recognized species, with new species continually being discovered and described.
- Taxonomic classification is based on morphological characteristics, genetic analyses, and biochemical traits.

III. Morphology and Growth Characteristics:

- Penicillium species are characterized by their filamentous hyphae, which form a network known as mycelium.
- Conidiophores, specialized structures for asexual reproduction, produce conidia (asexual spores) in chains or clusters.
- Colonies of Penicillium exhibit various colors (e.g., green, blue, yellow) due to the production of pigmented conidia.
- Optimal growth conditions typically include moderate temperatures (20-30°C), slightly acidic to neutral pH, and high humidity.

IV. Habitat and Ecology:

- Penicillium species are ubiquitous in nature, found in diverse habitats ranging from soil and decaying plant matter to indoor environments such as homes and food processing facilities.
- Some species have adapted to extreme environments, including high-salt environments, acidic soils, and cold climates.
- Certain Penicillium species form symbiotic relationships with plants, aiding in nutrient uptake and disease resistance.

V. Economic and Biotechnological Importance:

- Penicillium species have significant economic importance in various industries, including food, pharmaceuticals, agriculture, and biotechnology.
- They are used in the production of various fermented foods and beverages, such as cheese, soy sauce, and fermented meats.
- - Some species produce enzymes with industrial applications, including amylases, proteases, and cellulases, used in bioremediation, biofuel production, and textile processing.

VI. **Medical and Pharmaceutical Relevance:**

- Certain Penicillium species are sources of important antibiotics, including penicillin, the first antibiotic discovered by Alexander Fleming in 1928.
- Penicillin and its derivatives revolutionized medicine and remain essential in the treatment of bacterial infections.

VII. **Antimicrobial Potential:**

- Penicillium species are known for their antimicrobial properties, producing compounds that inhibit the growth of bacteria, fungi, and other microorganisms.
- Apart from penicillin, some species produce secondary metabolites with antimicrobial activity, including patulin, citrinin, and roquefortine.

VIII. **Research and Development:**

- Ongoing research focuses on exploring the biodiversity of Penicillium species, identifying novel bioactive compounds, and optimizing production processes.
- Biotechnological approaches, such as genetic engineering and fermentation optimization, aim to enhance the yield and efficacy of antimicrobial compounds derived from Penicillium.
- Understanding the ecological roles and evolutionary relationships of Penicillium species informs conservation efforts and the sustainable utilization of fungal resources.

2) ***Aspergillus nige***

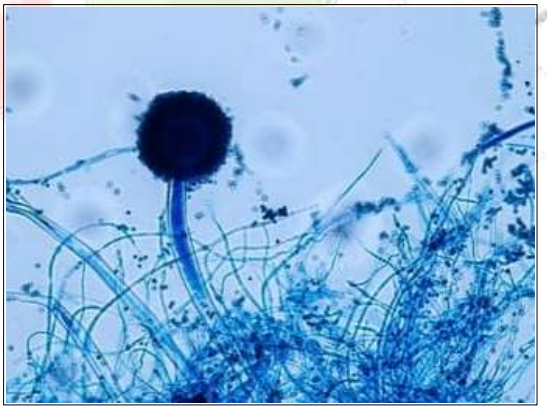

Fig.no.4 : ***Aspergillus Niger***

I. General Information:

- Aspergillus niger is a filamentous fungus belonging to the genus Aspergillus, which encompasses a diverse group of molds commonly found in soil, decaying vegetation, and indoor environments.
- It is one of the most widely studied and utilized species within the genus due to its significance in industry, biotechnology, and research.

II. Taxonomy and Classification:

- Aspergillus niger belongs to the Kingdom Fungi, Phylum Ascomycota, Class Eurotiomycetes, Order Eurotiales, and Family Aspergillaceae.
- Its taxonomic classification includes the species name "niger," which refers to its characteristic black color of the conidial mass.

III. Morphology and Growth Characteristics:

- Aspergillus niger exhibits typical fungal morphology, characterized by septate hyphae and conidiophores.
- Conidiophores produce conidia (asexual spores) in large, dense heads, giving the colonies a black appearance.
- It grows rapidly on various substrates under aerobic conditions and thrives in acidic environments.

IV. Habitat and Ecology:

- Aspergillus niger is cosmopolitan in distribution and can be found in diverse habitats, including soil, decaying organic matter, compost piles, and indoor environments such as air conditioning units and damp buildings.
- It plays a role in the decomposition of organic material and nutrient recycling in ecosystems.

V. Economic and Biotechnological Importance:

- Aspergillus niger has significant economic importance in various industries, including food and beverage, agriculture, biotechnology, and pharmaceuticals.
- It is used in the production of citric acid through fermentation of carbohydrates, making it one of the primary industrial applications of the fungus.
- Additionally, Aspergillus niger is employed in the production of enzymes, organic acids, bioactive compounds, and other biotechnological products.

VI. Medical and Pharmaceutical Relevance:

- While Aspergillus niger is generally considered non-pathogenic to humans, it can cause opportunistic infections, particularly in immunocompromised individuals.
- Infections caused by Aspergillus niger are rare but may lead to invasive aspergillosis, a serious condition with high mortality rates.
- The fungus also produces mycotoxins, such as ochratoxin A, which can contaminate food and feedstuffs, posing risks to human and animal health.

VII. Antimicrobial Potential:

- Aspergillus niger and its secondary metabolites exhibit antimicrobial activity against a wide range of microorganisms, including bacteria, fungi, and viruses.
- Some compounds isolated from Aspergillus niger have shown promising antimicrobial properties and are being explored for potential therapeutic applications.

VIII. Research and Development:

- Research on Aspergillus niger spans various disciplines, including microbiology, biotechnology, genetics, and pharmacology.
- Ongoing studies focus on elucidating the genetic and metabolic pathways of Aspergillus niger, optimizing fermentation processes for industrial applications, and exploring its biotechnological and pharmaceutical potential.

3) *Candida albicans*

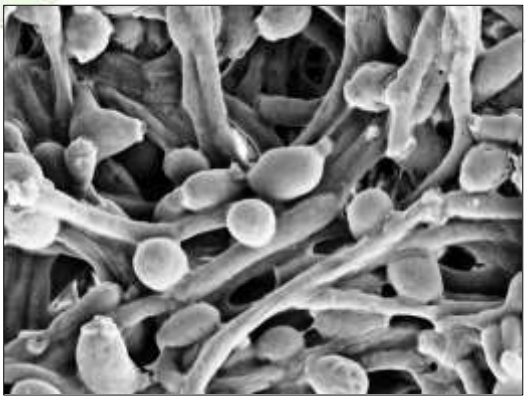

Fig.no.5 :*Candida Albicans*

I. General Information:

- Candida albicans is a species of yeast, belonging to the fungal kingdom.
- It is one of the most common human fungal pathogens, capable of causing opportunistic infections in immunocompromised individuals.
- Candida albicans is part of the normal microbial flora in the human body, commonly found in the gastrointestinal tract, oral cavity, and genitourinary tract.

II. Taxonomy and Classification:

- Candida albicans belongs to the kingdom Fungi, phylum Ascomycota, class Saccharomycetes, order Saccharomycetales, and family Saccharomycetaceae.
- It is classified under the genus Candida, which comprises numerous species of yeast-like fungi.

III. Morphology and Growth Characteristics:

- Candida albicans typically appears as oval-shaped budding yeast cells under a microscope.
- In addition to yeast cells, it can also form elongated hyphal structures under certain environmental conditions, contributing to its pathogenicity.
- It grows well on various laboratory culture media, including Sabouraud dextrose agar, at temperatures ranging from 20°C to 37°C.

IV. Habitat and Ecology:

- Candida albicans is a commensal organism found in the mucosal surfaces of humans and other warm-blooded animals.
- It thrives in moist and warm environments, such as the oral cavity, gastrointestinal tract, vaginal tract, and skin folds.
- While it is typically a commensal, Candida albicans can become pathogenic under conditions of host immunodeficiency or dysbiosis.

V. Economic and Biotechnological Importance:

- Candida albicans has both positive and negative economic impacts. It is used in biotechnological processes such as fermentation for the production of various compounds, including enzymes and pharmaceuticals.
- However, it is also a common cause of healthcare-associated infections, leading to increased healthcare costs and economic burden.

VI. Medical and Pharmaceutical Relevance:

- Candida albicans is a significant human pathogen, causing a range of infections, including oral thrush, vaginal yeast infections, and systemic candidiasis.
- In immunocompromised individuals, such as those with HIV/AIDS or undergoing chemotherapy, Candida albicans infections can be life-threatening.
- It poses challenges in clinical settings due to its ability to develop resistance to antifungal agents, leading to treatment failures.

VII. Antimicrobial Potential:

- Despite its pathogenicity, Candida albicans also exhibits potential antimicrobial properties.
- Some studies have explored the use of Candida albicans-derived compounds as antimicrobial agents against other pathogens, highlighting its biotechnological potential.

8. Research and Development:

- Candida albicans remains a subject of extensive research, particularly in the fields of microbiology, immunology, and infectious diseases.
- Efforts are ongoing to better understand its virulence mechanisms, host-pathogen interactions, and mechanisms of antifungal resistance.
- Research into novel antimicrobial agents, including natural products and synthetic compounds, continues in response to the growing threat of Candida albicans infections.

4) *Pseudomonas aeruginosa :*

Fig.no.6 : *Pseudomonas Aeruginosa*

I. General Information:

- *Pseudomonas aeruginosa* is a Gram-negative, rod-shaped bacterium belonging to the genus Pseudomonas.
- It is classified as an opportunistic pathogen, meaning it primarily causes infections in individuals with compromised immune systems or underlying health conditions.
- *P. aeruginosa* is known for its remarkable adaptability and resistance to antibiotics, posing significant challenges in clinical settings.

II. Taxonomy and Classification:

- - Domain: Bacteria
- - Phylum: Proteobacteria
- - Class: Gammaproteobacteria
- - Order: Pseudomonadales
- - Family: Pseudomonadaceae
- - Genus: Pseudomonas
- - Species: Pseudomonas aeruginosa

III. Morphology and Growth Characteristics:

- - *Pseudomonas aeruginosa* typically appears as straight or slightly curved rods under the microscope.
- - It is motile due to the presence of polar flagella.
- - *P. aeruginosa* is aerobic and grows well under aerobic conditions, forming smooth, round colonies on agar plates.
- - The bacterium produces a characteristic fruity or grape-like odor.

IV. Habitat and Ecology:

- *Pseudomonas aeruginosa* is ubiquitous in nature and can be found in various environments, including soil, water, and vegetation.
- It thrives in moist environments and is commonly found in hospital settings, particularly in water sources, sinks, and medical equipment.
- *P. aeruginosa* can also colonize the respiratory and gastrointestinal tracts of humans and animals.

V. Economic and Biotechnological Importance:

- *Pseudomonas aeruginosa* plays a role in bioremediation by degrading a wide range of organic compounds, including hydrocarbons and pollutants.
- Some strains of *P. aeruginosa* are used in biotechnological applications, such as the production of enzymes and biosurfactants.

VI. Medical and Pharmaceutical Relevance:

- *Pseudomonas aeruginosa* is a leading cause of healthcare-associated infections, including pneumonia, urinary tract infections, bloodstream infections, and wound infections.
- Infections caused by *P. aeruginosa* are often difficult to treat due to its intrinsic resistance to many antibiotics and its ability to develop resistance mechanisms.

VII. Antimicrobial Potential:

- Despite being a formidable pathogen, *Pseudomonas aeruginosa* has garnered attention for its potential as a source of antimicrobial compounds.
- Some strains of *P. aeruginosa* produce secondary metabolites with antimicrobial properties, which are being investigated for their potential in drug discovery.

VIII. Research and Development:

- Ongoing research focuses on understanding the virulence factors and mechanisms of antibiotic resistance in *Pseudomonas aeruginosa.*
- Efforts are underway to develop new antimicrobial agents and alternative treatment strategies to combat infections caused by this pathogen.

3.0 Aim and Objective

Aim :-

Evaluation of antimicrobial activity of leaves and stems of *cocculus hirsutus.*

Objective :-

1. Assess the ability of Cocculus hirsutus ethanolic extract to inhibit the growth of test microorganisms.

2. Compare the antimicrobial efficacy of the extract to the standard antibiotic drug, gentamycin.

3. Identify the most effective concentrations of the Cocculus hirsutus extract for antimicrobial activity.

4. Literature Review :

Fayiah, M., Fayiah *et al.*(2023) : The use of herbal medicine has the ability to promote sustainable development and have a variety of effects on societal livelihoods. The purpose of this evaluation is to provide decision-makers and healthcare authorities with up-to-date information on the benefits of herbal medicine in achieving the sustainable development goal of providing everyone with access to quality healthcare by 2030. This chapter will give a general summary of how herbal medicine might support sustainable development in isolated communities. As a result, the chapter examined and culled significant data on sustainable development topics from numerous sources. The overview outlines the most prevalent kinds of herbal medications used to cure ailments, as well as their side effects, difficulties, and future directions for gaining international acceptance for herbal treatments. But today's increasing need for herbal medication is significantly boosting.[1]

Dr. Susan Sam *et al.*(2018) : This paper attempts to analyse the importance and effectiveness of Herbal and Traditional medicines. It is a medical system that has its origin in ancient cultures and that involves the medicinal use of plants and its extract to treat illness and to assist bodily functions. It has been around since prehistoric times. Herbal medicine is still the mainstay of about 75% of the world population, especially in the under developed and developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. However, in the last few years there has been a major increase in their use in the developed world. In Germany and France, many herbs and herbal extracts

are used as prescription drugs. Herbal treatments are the most popular form of traditional medicine, and are highly lucrative in the international marketplace. The medicinal plants contribute to cater 80% of the raw materials used in the preparation of drug. It can be taken orally or applied locally.[2]

Logesh, Rajan *et al*.(2020):It is a perennial climber that's primarily found in subtropical and tropical regions. This article's primary goal is to gather and evaluate scientific data about pharmacological effects, bioactive chemical components, and traditional usage. Techniques: The online bibliographic databases (e.g., MEDLINE/PubMed, SciFinder, Web of Science, Google Scholar, and Scopus) were used to get scientific data on C. hirsutus. Books and proceedings were among the secondary sources from which information about traditional uses was also gathered. Findings: It has been claimed that some C. hirsutus plant components are used to cure fever, skin conditions, stomach problems, and bladder infections. Whole plants or various plant parts have been reported to include alkaloids such as jasminitine, hirsutine, and cohirsitine, as well as their derivatives, a few flavonoids, triterpene derivatives, and volatile chemicals.[4]

AG Fahmy, El-Bakry *et al*.(2011) : The presence of a plant that adds a significant new record to the Flora of Egypt was discovered during the botanical survey of Wadi Wateer, which is situated in the southeast of Sinai: Cocculus hirsutus (L.) Theob. The Egyptian flora has not before documented this plant in its natural state. The Saharo-Sindian phytogeographical element's plant taxa make up the majority of the vegetation in Wadi Wateer. Acacia tortilis (Forssk.) Hayne subsp. raddiana (Savi) Brenan, Chrozophora brocchiana Vis., Lycium shawii Roem & Schult., Moricandia sinaica (Boiss.) Boiss., Cocculus hirsutus (L.) Theob., and Cocculus pendulus (J.R. & G. Forst.) Diels are among the numerous plants in the wadi that have been identified as belonging to the Sudanian chorotype. The existence of chorotype plant species from Sudan, such as the recently discovered Cocculus hirsutus. [22]

5. MATERIALS AND METHODS

5.1 Instrument and Chemicals:

Apparatus and Instrument: - Thermostatic water bath, autoclave,incubator, heating mantle, Test Tubes, Beakers, Petri plates , Soxhlet apparatus ,etc

Chemicals: - Distilled water, ethanol, DMSO ,moller hinton agar ,potato dextrose agar, Mayer's reagent, Dragendroff's reagent, Hager's reagent, Molish's reagent, Fehling's A and B solutions, Benedict's reagent, Conc.H2SO4,gentamycin.

5.2 Plant collection -

The leaves and stems of Cocculus hirsutus were collected from around area of chinchewadi village, gadhinglaj, (Kolhapur) in the month of february. Its identity was confirmed from the department of Botany, Dr. V.A.Sardesai ,Shivraj college , Gadhinglaj. (SCG/05/2024/25) & (SCG/09/2024/25)

5.3 Preparation of plant extract :-

The fresh leaves and stems of Cocculus hirsutus were thoroughly washed under tap water and then dried in shade for a few weeks. After drying, they were crushed into a coarse powder. 60 g of the dried powder were then extracted for 24 hours using 300 ml of solvent (99% ethanol). Repeated extraction was carried out with the same solvent until a colorless solvent was obtained. The condensed extract was utilized for screening primary metabolites. Soxhlet equipment was employed in this study, where powdered plant material (60 g) was extracted with organic solvents (300 ml), such as ethanol, in a Soxhlet apparatus.

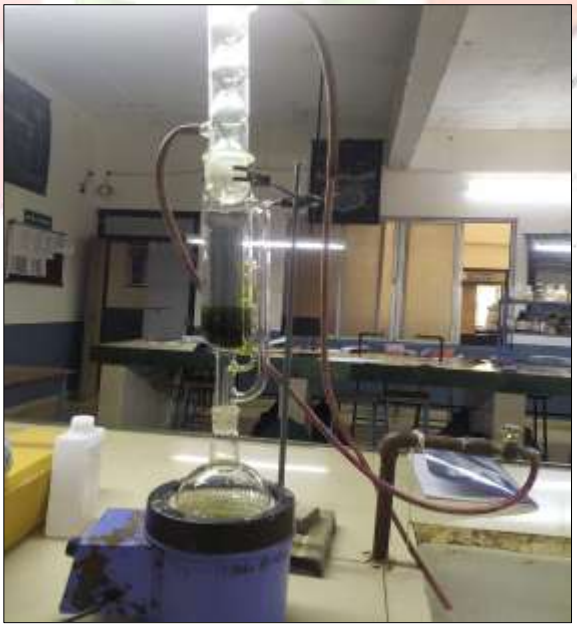

Fig.no.7 : Extraction

5.4 Phytochemicals Screening

	S. Metabolites	Name of Test	Results
1	Alkaloids	- Mayer's Test (Evans, 1997) - Wagner's Test (Wagner, 1993) -Dragendroff's Test - Hager's Test (Wagner et al., 1996)	+ + + +
2	Tannins and Phenols	- Ferric Chloride Test (Mace, 1963) - Lead Acetate Test	- +
3	Flavonoids	- Alkaline Test	+
4	Saponins	- Froth Test	+
5	Terpenoids	- Horizon's Test	-
6	Glycosides	- Legal Test	+

Table NO.2:Chemical Tests

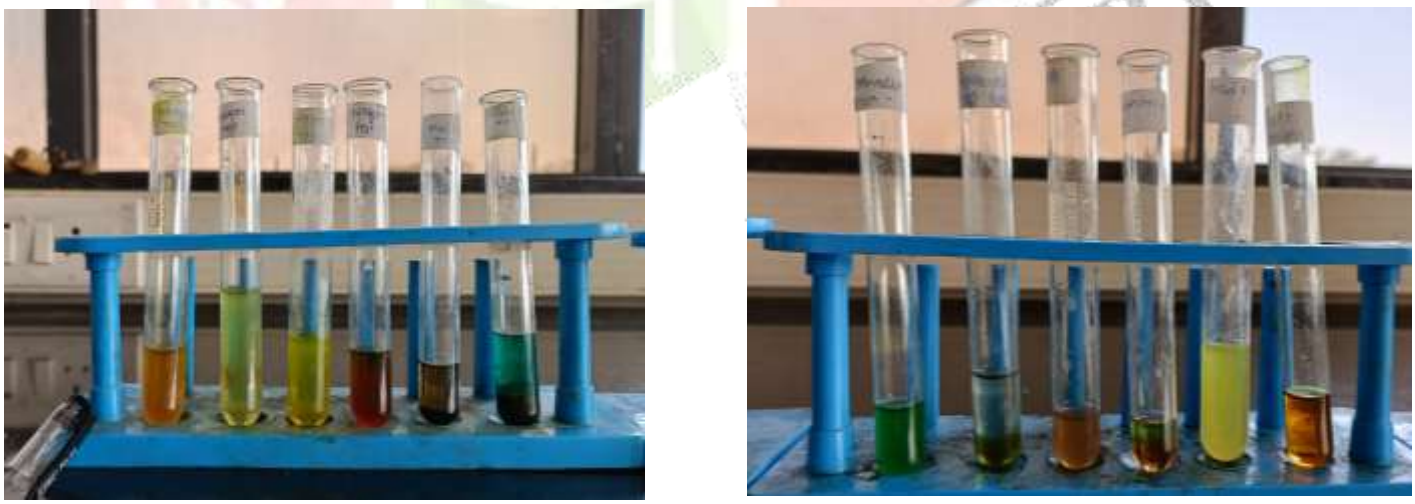

Fig.no.8:Chemical tests

6. Microbe and Media :

The clinical bacterial isolates such as *Pseudomonas Aerogenosa* and clinical fungal isolates such as *penicillium , Candida albicans,,Aspergillus Niger* were used. The bacterial and fungal isolates were obtained from Dr. Ghali college, Gadhinglaj and the slants were maintained in nutrient agar and potato dextrose agar respectively for bacterial and fungal isolates, which were stored at 4^0C.

6.1 Dilutions:

1) dilution for c.hirsutus extract = 1gm/10 ml DMSO

=0.1gm/ 1ml

(25 µl,50 µl,75 µl, and 100 µl)

2) dilution for Gentamycin = 8mg/10ml (dist. water)

(0.125 µl)

6.2 Procedure for antibacterial

Prepare Moller Hinton agar

↓

At Molten stage add suspension of test organism

↓

Shake well and add into petry plate

↓

After solidifying of agar, make a wells by using cork borer

↓

In the well , add a dilutions of cocculus hirsutus extract are as 25 µl,50 µl,75 µl, and 100 µl in each well and as well as for control add DMSO and for standard add 125 l Gentamycin

↓

after adding of dilutions . Plates are kept in deep freezer for 1 hour for the diffusion process

↓

After diffusion , plates were transfered in incubator at 37^0C for 24 hours

↓

After a incubation zone of inhibition was observed

6.3 Procedure for Antifungal :

Prepare a potato dextrose agar

↓

At a molten stage ,add suspension of test organism in each 20 ml separately

↓

Shake well and pour in petry plate

↓

After solidifying make a wells by using cork borer

↓

In the well , add the dilution of cocculus hirsutus exract are as 25 µl, 50 µl,75 µl and 100 µl in each well respectivly and for control add DMSO and for standard add Gentamycin in well

↓

Then the plate are kept in deep freezer for 1 hour and after 1 hour . Plates are transfered in incubator at 25^0C or room temperature for 24 to 48 hours

↓

After incubation , plates are observed for zone of inhibition

7. Results and Discussion:

Organisms		Ethanolic extract of cocculus hirsutus				Gentamycin
		25 µl	50 µl	75µl	100 µl	
1	*Penicilium*	15 mm	22mm	26mm	32mm	40mm
2	*Aspergillus Niger*	20mm	25mm	32mm	37mm	36mm
3	*Candida Albican*	17mm	22mm	30mm	35mm	41mm
4	*Pseudomonas Aerogenosa*	13mm	20mm	30mm	32mm	45mm

Table no.3: Antimicrobial activity of extracts of the plant *Cocculus hirsutu*

The results indicate that *Cocculus Hirsutus* extract exhibits notable antimicrobial activity against all tested microorganisms, although it is generally less effective than the standard drug Gentamycin.

When compared to standard antibiotics (Gentamycin), *Cocculus Hirsutus* extracts showed moderate activity, with ethanolic extract being the most effective but still less potent than standard antibiotics.

The ethanolic extract of *Cocculus Hirsutus* showed significant antibacterial activity against all tested strains, particularly *Pseudomonas Aerogenosa.*

Similarly, the ethanolic extract exhibited noteworthy antifungal activity, especially against *Penicilium , Aspergillus Niger and* Candida albicans. The presence of phenolic compounds might be responsible for disrupting fungal cell membranes and inhibiting their growth.

Fig.no.9 : Results of antimicrobial activity

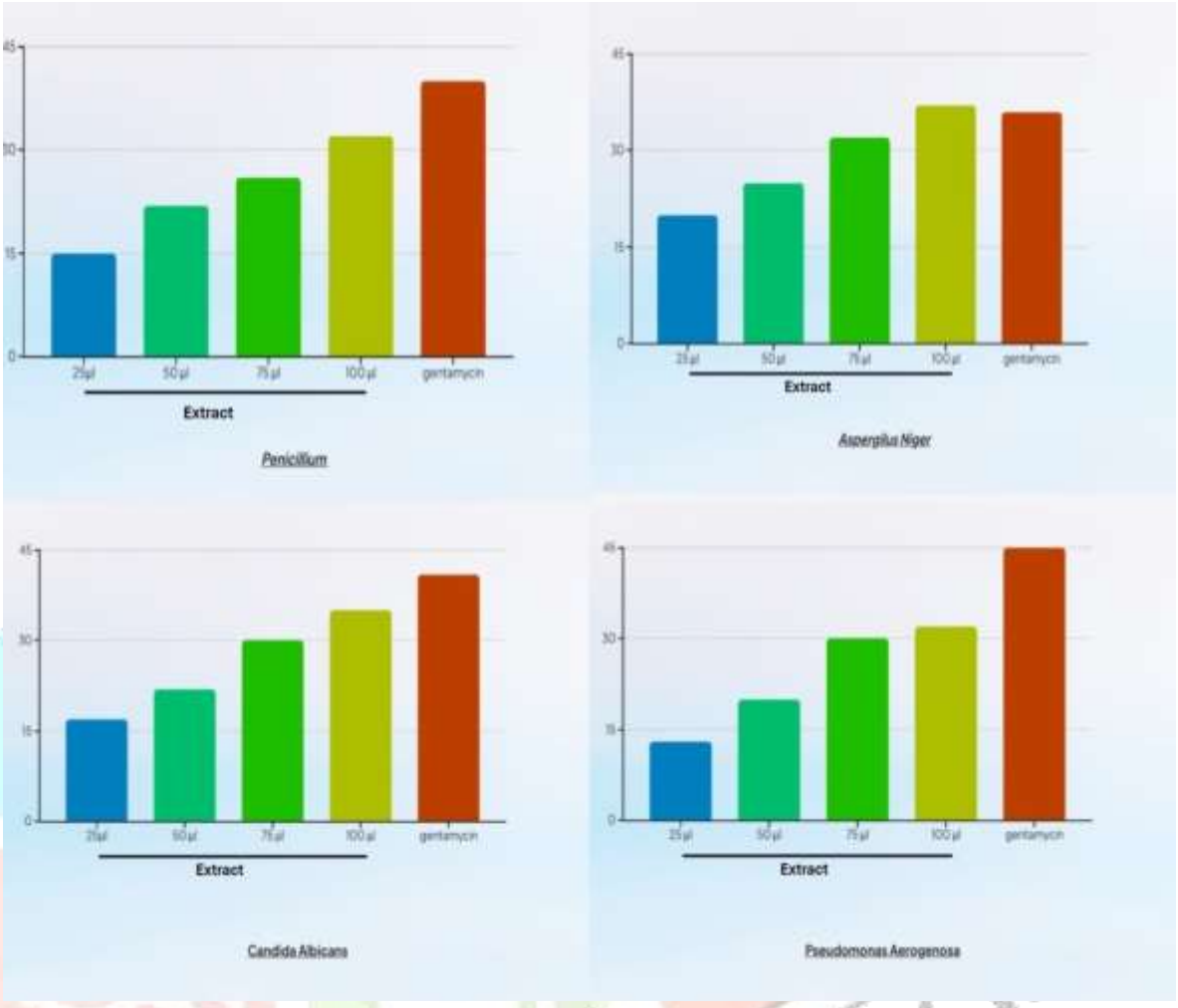

Fig.no.10: Graphical Representation

8. Conclusion:

- The study aimed to evaluate the antimicrobial efficacy of Cocculus Hirsutus, which has been traditionally used in herbal medicine. The presence of bioactive compounds such as alkaloids, flavonoids, and tannins in the extracts likely contributes to the antimicrobial properties observed.
- Cocculus Hirsutus possesses antimicrobial properties against a range of microorganisms, including bacteria and fungi. However, its efficacy is lower compared to the standard antibiotic Gentamycin. Further studies could focus on isolating and identifying specific active compounds within Cocculus Hirsutus and exploring synergistic effects when combined with other antimicrobial agents to enhance its potency.
- Cocculus Hirsutus exhibits promising antimicrobial properties, particularly when extracted with ethanol, making it a potential candidate for developing natural antimicrobial agents against various pathogenic microorganisms.
- Further research and development could harness its potential as an alternative or complementary antimicrobial agent.

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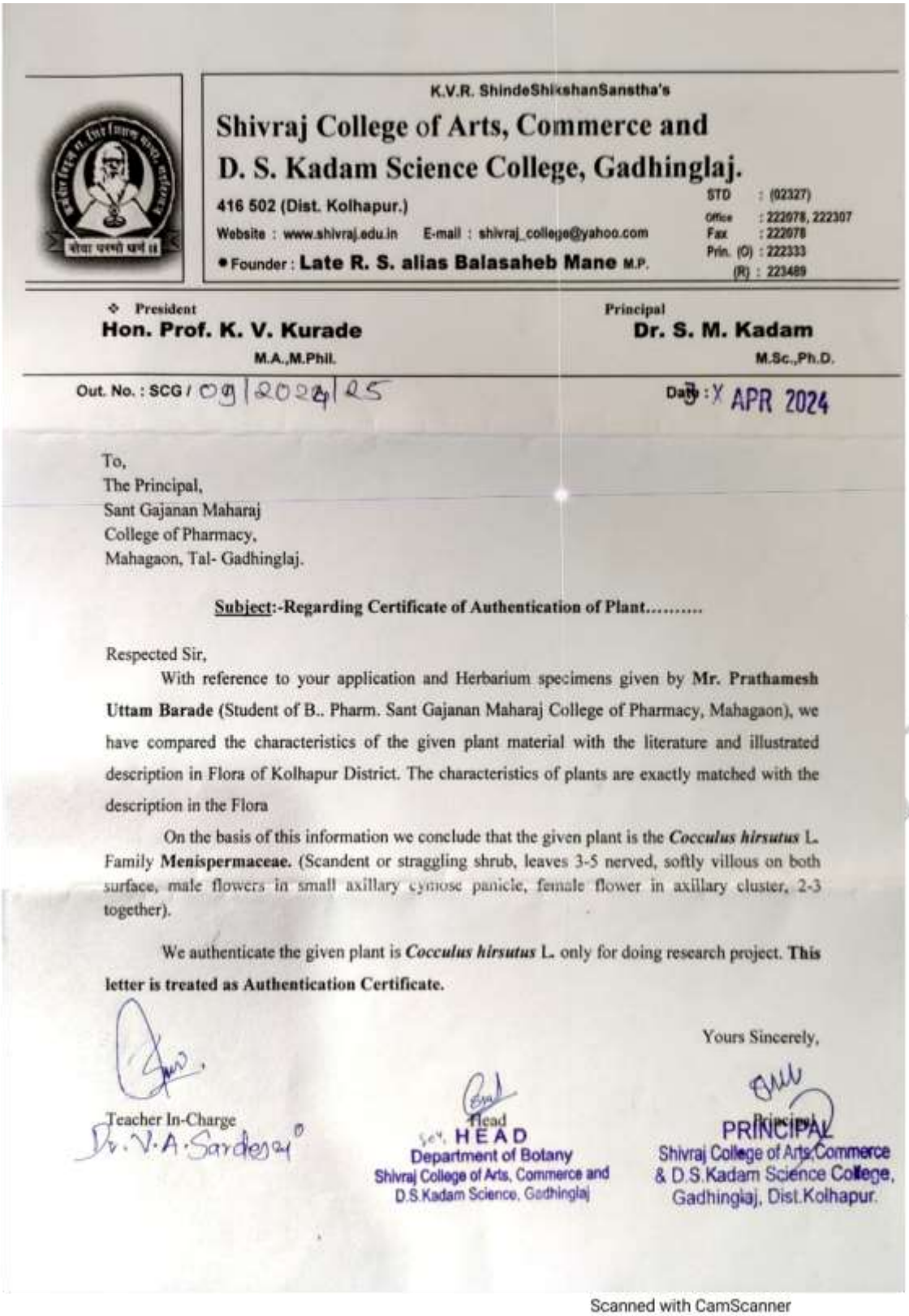

K.V.R. ShindeShikshanSanstha's

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❖ President
Hon. Prof. K. V. Kurade
M.A.,M.Phil.

Principal
Dr. S. M. Kadam
M.Sc.,Ph.D.

Out. No. : SCG / 09 / 2024 / 25 Date : 5 APR 2024

To,
The Principal,
Sant Gajanan Maharaj
College of Pharmacy,
Mahagaon, Tal- Gadhinglaj.

Subject:-Regarding Certificate of Authentication of Plant.....

Respected Sir,

With reference to your application and Herbarium specimens given by **Mr. Prathamesh Uttam Barade** (Student of B.. Pharm. Sant Gajanan Maharaj College of Pharmacy, Mahagaon), we have compared the characteristics of the given plant material with the literature and illustrated description in Flora of Kolhapur District. The characteristics of plants are exactly matched with the description in the Flora

On the basis of this information we conclude that the given plant is the ***Cocculus hirsutus*** L. Family **Menispermaceae.** (Scandent or straggling shrub, leaves 3-5 nerved, softly villous on both surface, male flowers in small axillary cymose panicle, female flower in axillary cluster, 2-3 together).

We authenticate the given plant is ***Cocculus hirsutus*** L. only for doing research project. **This letter is treated as Authentication Certificate.**

Yours Sincerely,

Teacher In-Charge
Dr. V. A. Sardesai

Head
HEAD
Department of Botany
Shivraj College of Arts, Commerce and
D.S.Kadam Science, Gadhinglaj

Principal
PRINCIPAL
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Principal
Dr. S. M. Kadam
M.Sc.,Ph.D.

Out. No. : SCG / 05 / 2024 / 25

Date : 3 APR 2024

To,
The Principal,
Sant Gajanan Maharaj
College of Pharmacy,
Mahagaon, Tal- Gadhinglaj.

Subject:-Regarding Certificate of Authentication of Plant.....

Respected Sir,

With reference to your application and Herbarium specimens given by **Mr. Harshad Laxman Mitke** (Student of B.. Pharm. Sant Gajanan Maharaj College of Pharmacy, Mahagaon), we have compared the characteristics of the given plant material with the literature and illustrated description in Flora of Kolhapur District. The characteristics of plants are exactly matched with the description in the Flora

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