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Microbiological Activity of *Eclipta alba, Tridax procumbens, Allium controversum, Lawsonia inermis* in Different Solvents and Sterility Testing of Commercially Available Pharmaceutical Products

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ABSTRACT

The investigation of alternative antimicrobial agents derived from natural sources is vital due to the growing prevalence of bacteria that are resistant to antibiotics. The antibacterial activity of methanolic extracts of Lawsonia inermis (henna), Tridax procumbens (coat buttons), Eclipta alba (false daisy), and Allium controversum (garlic) against human pathogenic bacteria was assessed in this study. The agar well diffusion method was used to test the extracts against Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus. According to the findings, every extract shown notable antibacterial activity, with Allium sativum showing the strongest activity against every bacterium that was tested. The fundamental idea behind a sterility test is that if microorganisms are introduced into a transparent media that promotes their growth (i.e., the ideal conditions for temperature, pH, nutrition, and water),If the test is conducted in an uncontaminated environment, a change in the medium's turbidity can indicate the presence of microorganisms. Since every container in a batch is identical, the test interpretation is predicated on this fact. This study highlights the significance of maintaining pharmaceutical product sterility in order to prevent microbiological contamination and guarantee patient safety, as well as the potential of these plant extracts as natural antibacterial agents.

Keywords : salmonella typhi, staphylococcus aureus, escherichia coli, allium sativum, eclipta alba, lawsonia inermis, tridax procumbens

INTRODUCTION

The following are some classic crude medication extracts that have been studied for their antimicrobial activity:

- **Eclipta Alba:**

Alternatives: bhangra, babri, and false daisy Eclipta Alba (L)

Hassk is its botanical name, and it prostrates Roxb.

The Asteraceae family

Geographical Source: Nepal, Brazil, China, and India.

Ecliptin, ecliptal, triterpen, ecliptabine, amyrin, phenolic acids, alkaloids, flavonoids, polyacetylenes, thiopenes, cumentans, β-terthienylmethanol, wedelolactone, and sterols are among the chemical constituents.

Therapeutic and Medicinal Uses:

1. **Antimicrobial activity:** Bhringaraj shown efficacy against the tested bacterial species. Numerous illnesses, including typhoid, food-borne infections, oral and throat sores, nosocomial infections, and acne, can be treated with this. The idea of using this plant to create a medication that may be consumed by humans, potentially to cure bacterial infections, has been made possible by this work.

2. **Hair Health:** Bhringraj is well known for preventing early graying, boosting hair growth, and decreasing hair loss. It is utilized in powders and oils that are applied straight to the scalp.

3. **Liver Health:** It is used to detoxify the liver and enhance general liver function because it is thought to have hepatoprotective (liver-protecting) qualities.

4. **Skin Care**: Bhringraj is also utilized in some skin care regimens. like irritation, dermatitis, and acne.

5. **Anti-inflammatory & Antioxidant**: The plant has substances that can lower inflammation and offer antioxidant advantages, assisting in defending the body against harm from free radicals.

6. **Anti-Aging:** The herb's revitalizing qualities, which support the maintenance of youthful vitality and energy, make it a great anti-aging therapy.

7. **Digestive Health**: Indigestion, constipation, and bloating are among the digestive problems that it is occasionally used to treat.

- **Chrysanthemum procumbens:**

Synonyms: Balbisa pendunculata, Amellus pedunculatus Ortega ex wild, Balbisa elongata

Botanical name: Tridax Procumbens

Family: Asteraceae

Medicinal and Therapeutic uses:

Antibacterial activity

The entire Tridax plant has antimicrobial properties. Two Gram-positive (Bacillus subtilis, Staphylococcus) and two Gram-negative (Escherichia coli, Pseudomonas aeruginosa) bacteria are present in this entire plant extract. Using the disc diffusion method, this study demonstrated the potent antibacterial activity of the entire Tridax plant exclusively against Pseudomonas aeruginosa. Tridax procumbens leaf extract is used to analyze antibacterial activity utilizing a variety of solvents, including ethanol, ehylacetate and chloroform. Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, were all tested with antibiotics using the disc diffusion method. The greater zone of inhibition was seen in gram-negative bacteria. Various solvent extracts were used to test for antibacterial activity.

Anti fungal Activity

When Tridax procumbens leaf extract was tested against Fusarium oxysporum, the results demonstrated good antifungal activity. Candida albicans and Candida tropicalis were found to be susceptible to the methanol leaf extract of Tridax procumbens L. Tridax procumbens extract will eventually be used as an efficient treatment for Candida infections.

Hypotensive effect

Effect of hypotension Tridax procumbens leaf's hypotensive impact was examined. significantly lower mean arterial blood pressure in dose-dependent ways. A considerable decrease in heart rate is indicated by the greater dose, however no changes were observed with the lesser amount. According to reports, Tridax procumbens Linn. Leaves have a hypotensive.

Immunomodulatory effect

When administered to Albino rats, the ethanol leaf extract of Tridax has been shown to exhibit immunomodulatory effects and to suppress the growth of Pseudomonas aeruginosa. An aqueous extract of Tridax procumbens was examined for immunomodulatory properties.

Anti diabetic activity

Tridax procumbens Linn. Leaves have antidiabetic properties that cause a substantial drop in blood glucose levels when extracted with alcohol and water.

Anti inflammatory activity

Tridax procumbens Linn. Leaves have Anti inflammatory activity.

Wound healing

The intricate and ever-changing process of wound healing can repair tissue layers and cellular systems. Water extract from the entire Tridax procumbens L. plant can restore normal wound healing in rats with weakened immune systems.

- **Allium controversum:**

Allium arenarium and Allium controversum are synonyms.

The biological source is the bulb of the "Allium sativum,"

a member of the "Liliaceae" family.

Geographical source: Mainly grown in Southeast Asia, India, and Italy

Chemical components **:**

diallylthiosulfinate, or allicin

Non volatile compounds:

g-glutamyl-salk(en)yl-L-cysteine, specifically g-glutamyl-s-allyl-L-cysteine, g-glutamyl-s-trans-1-propenyl-L-cysteine, and salt(en)yl-L-cysteinesulfoxide, including s-allyl-L-cysteine sulfoxide (alliin), s-(trans-1-propenyl)-L-cysteine sulfoxide (isoallin), and S-methyl-L-cysteine sulfoxide (methiin), with a trace amount of S-allylcysteine (SAC).

volatile compounds

Ajoene, vinyl dithiins (2-vinyl-1-3-dithiin, 3-vinyl-2-dithiin), diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), methyl allyl disulfide (MADS), and methyl allyl sulfide are examples of volatile compounds.

Medicinal uses:

Cardiovascular health:

Garlic lowers triglyceride and cholesterol levels, which lowers the risk of heart disease.

Antibacterial and antiviral qualities: Research has demonstrated that garlic possesses antibacterial and antiviral qualities, which enable it to effectively combat a variety of infections.

Antioxidant qualities: Antioxidants found in garlic help prevent cell damage and lower the chance of developing certain illnesses.

Cancer prevention: Studies have demonstrated the anti-cancer effects of garlic, especially in lowering the incidence of colorectal and stomach cancers.

Immune system support: Research has demonstrated that garlic contains immunomodulatory properties that aid in immune system support.

Therapeutic uses

1. **Digestive problems**: Bloating, gas, and diarrhea are among the digestive problems that garlic has been used to cure.

2. **Respiratory problems**: Bronchitis, asthma, and allergies are among the respiratory conditions that garlic has been used to treat.

3. **Skin conditions**: Psoriasis, eczema, and acne have all been treated with garlic.

4. **Wound healing:** Garlic has been used to lower the risk of infection and encourage wound healing.

5. **Parasite control**: Tapeworms and hookworms are two parasite illnesses that garlic has been used to cure.

- **Lawsonia inermis :**

Henna, Casearia multiflora, and Alcanna spinosa are synonyms.

Lawsonia inermis is its botanical name.

Geographical sources include Asia, Africa, Australia, and India.

Chemical Components: Lawsone Lawsonia inermis

Therapeutic Applications

1. **Skin and hair care**: For millennia, henna has been used to support healthy skin and hair.

2. **Wound healing**: Henna has been used to lower the risk of infection and encourage wound healing.

3. Henna is useful against a variety of inflammatory disorders because of its **anti-inflammatory qualities.**

4. **Antibacterial and antifungal qualities:** Research has demonstrated that henna possesses antibacterial and antifungal qualities, which enable it to effectively combat a variety of ailments.

5. **Cancer prevention:** Research has demonstrated the anti-cancer effects of henna, especially in lowering the incidence of skin cancer.

- **Definition of sterility**

A microbiological technique called sterility testing determines whether a product is tainted by microbes. It is employed to guarantee that items that are meant to be sterile are devoid of living germs.

Sterility testing technique

• A variety of sterility testing methods are available, primarily two kinds.

Direct inoculation

Here, the test article is directly inoculated with two different types of media to determine the presence of both aerobic and anaerobic organisms.

Membrane filtration

Here, the test sample and standard preparation are simultaneously filtered via two membrane filters. The samples are then incubated for 14 days starting on the test day, and the visibility of the aerobic and anaerobic microorganisms is finally checked.

METHODS AND MATERIAL:

Extraction of plant material

Plant matter is gathered. The plant material was thoroughly washed with distilled water and then allowed to dry for a week in the shade.

Maceration

Menstruum is poured all over the surface of finely ground drug material, like leaves. The container is then sealed and kept for three days. The contents are regularly swirled and, if in a bottle, shaken to ensure full extraction. After extraction, the micelle is filtered or decanted from the marc. The micelle is then evaporated in an oven or on top of a water bath to separate it from the menstrum.

The maceration technique has following advantages

Maceration is a simple process that doesn't call for any specialized tools or equipment.

• It is appropriate for certain substances that aren't very soluble in the solvent and only require prolonged contact with it; it doesn't require a trained operator.

• The proper strategy for less potent and less expensive medications.

MICROBIOLOGICAL ASSAY OF ANTIBIOTICS

Agar composition :

1.AGAR –15gm

2.BEEF EXTRACT-3gm

3.PEPTONE -5gm

4.NACL-5gm

5.WATER-1000ml

- **PREPARATION OF AGAR:**

To make agar, dissolve agar powder in distilled water, boil the mixture, and then pour it into containers.

Steps:

1. Find the weight of the agar.
2. Dissolve the agar in distilled water.
3. To achieve the same result, heat the mixture.
4. Reduce the mixture's temperature till it is comfortable.
5. Pour the mixture into containers.

6. Let the mixture cool fully

CYLINDER OR CUP PLATE METHOD

A previously liquified medium appropriate for the assay should be inoculated with the necessary quantity of the microorganism's suspension. The suspension should then be added to the medium, which should be between 40 and 50 degrees. To ensure that the medium layers are all the same thickness, place the big plates or petri dishes on a flat surface and immediately pour the infected medium into them until it reaches a depth of 3 to 4 mm.

PURE CULTURE TECHNIQUE(STREAK METHOD)

The streaking technique can be used to isolate a pure strain of a single species of microorganism, typically bacteria. The streak plate method technique consists of the following steps.

1. Remove one colony off a plate after sterilizing the loop. Place the inoculating loop over one quarter of the plate (area 1).

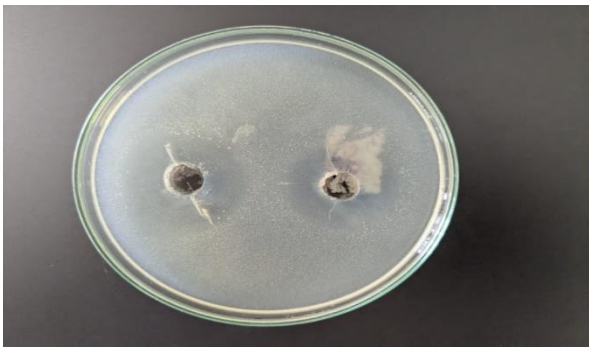
2. Continue the streaks into the second quarter of the plate (area 2) by repeating the sterile loop and going back to the area 1 edge.

3. Continue the streaks into the third quarter (area 3) of the plate by repeating the sterile loop and going back to the area 2 edge. By repeating the sterile loop and going back to the area 3 edge,the streaks are extended to the center fourth of the plate (area 4).

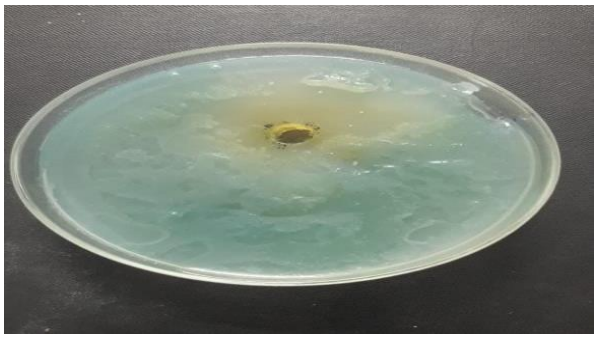
Results

Eclipta alba shows activity in Ethanol

Chrysanthemum procumbens shows activity in chloroform



Allium controversum shows activity in ethyl acetate



Lawsonia inermis shows activity in ethyl acetate

- **STANDARD SOLUTION PREPARATION- AMPICILLIN**

Commercially available Ampicillin injection contain 100mg of Amikacin in 2ml 1ml contains -50mg of Ampicillin

For preparing a stock solution 1mg/ml I'e 1000µg/ml

1. Take 1gm from the commercial sample & dilute 100ml with water gives 1mg/ml

2. From stock 1,1ml is taken and diluted to 10ml which gives stock 2 i. e 100 µg/ml

➢ **Sterility Testing Technique**

Direct Inoculation:

• The direct inoculation method of product sterility testing is collecting a sample and immediately introducing it to a suitable sterile culture medium. It is then incubated for a certain amount of time to look for any microbial growth, which could indicate contamination.

Key points about direct inoculation sterility testing

Sample preparation

• To guarantee adequate mixing with the culture medium, a tiny, aseptic sample of the ointment is collected; it might need to be diluted with a sterile diluent, such as isopropyl myristate.

Nutrient broth

• A wider variety of possible microbiological pollutants are addressed by the preparation of nutrient broth.

- **Preparation of nutrient broth:**
- **Composition**:

1. Beaf extract- 0.6gms
2. Peptone- 1gms
3. Sodium chloride- 1gm
4. Distilled water-200ml
5. PH- 7

Preparation of nutrient broth:

Steps

- Weigh the components above precisely.
- Fill a flask with distilled or deionized water and add the aforementioned components.
- Fully combine and dissolve the ingredients.
- Set the medium's pH to the appropriate level.
- Autoclave the broth for 15 to 25 minutes at 121°C to sterilize it.
- Let the broth cool down.
- Keep the broth colder than 25°C in a sterile flask or tube.

Inoculation process

prepared sample is sterilely introduced straight to the culture media.

Incubation process

give any existing bacteria enough time to proliferate, the inoculation culture media is incubated for a predefined amount of time, typically 14 days, at a controlled temperature.

CONCLUSION

According to our findings, the current investigation is valuable, particularly in regards to the antibacterial activity of the methanolic extract. A wide variety of bacteria were prevented from growing by the whole extract of Allium controversum. Tridax procumbens leaf ethanol extract has antimicrobial action since it stopped S. aureus and E. coli from growing. It was discovered that the ethanol extract was more effective against S. aureus and E. coli. Leaf extracts from Lwasonia inermis exhibit antibacterial action against the microorganisms that cause common skin illnesses. Eclipta abla exhibits anti-E. coli action.

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