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Review Article

**A COMPARATIVE ANALYSIS OF ANTI-BACTERIAL AND
ANTI-FUNGAL ACTIVITY OF POLY HERBAL
FORMULATION****A.Teja*, V. Naveen Kumar, M.Gowtham, Ganasala.Veera Gowtham, K. Sai Taruni.**Department of Pharmacology, Sri Siddhartha Pharmacy College,
Ammavarithota, Nuzvid, A.P**Abstract:**

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. So, the present study is to know that the efficiency of the herbal extracts on Microbial-activity and Anti-fungal activity (Nyctanthes arbortristis, Mentha piperita, salvadora persica). Estimation of Antimicrobial activity by Nutrient agar media, by the technique cup-plate method. In which all the extracts have antibacterial activity. Estimation of anti-fungal activity by potato dextrose agar media, by the inoculation method in which all the extracts have Anti-fungal activity.

Key Words: resistance, herbal extracts, Nyctanthes arbortristis, Mentha piperita, Salvadora persica, Agar media, cup-plate method, potato dextrose agar media, antifungal, antibacterial activity.

Corresponding author:

A. Teja *,
Department of Pharmacology,
Sri Siddhartha Pharmacy College,
Ammavarithota, Nuzvid. Mobile: 9133270945
Gmail: tejanaidu435@gmail.com

QR code



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INTRODUCTION:

Herbal formulations mean a dosage form consisting of one or more herbs or processed herbs in specified quantities to provide specific nutritional, cosmetic benefit for use to diagnose, treat, mitigate diseases of human beings or animals, alter the structure or physiology of human beings or animals. Herbal formulations contain an active substance or herbal substance or herbal preparation or herbal substance in combination with one or more herbal preparations. Herbal formulations are obtained by subjecting herbal substances to treatment such as extraction, distillation, expression, fractionation, purification, concentration or fermentation include comminuted or powdered. Different herbal formulations are tinctures, extracts, essential oils, expressed juices and processed exudates. Markers are chemically defined constituents or groups of constituents of an herbal substance, a herbal preparation or a herbal medicinal product which are of interest for control purpose independent of whether they possess any therapeutic activity. Markers serve to calculate the quantity of herbal substance or herbal preparation in the herbal formulations if the markers have been quantitatively determined in the herbal substance herbal preparations. Characterization of herbal formulations which includes are design and development, pharmacopeias tests and acceptance criteria, periodic testing, release, shelf-life acceptance criteria, In process tests, alternative procedures, evolving technologies, reference standard and statistical concepts.

EXAMPLES OF HERBAL FORMULATIONS:

These include powdered herbal substances, tinctures, extracts, essential oils expressed juices and processed exudates.

BENEFITS OF HERBAL MEDICINE:

Herbal medicines (HM) include herbs, herbal materials, herbal preparations and finished herbal products that contain as active ingredients parts of plants, or other plant materials, or combinations and are used especially for the prevention and treatment of Diseases. In contemporary times, HM remains a major component of the primary healthcare in many rural African and Asian communities. It also constitutes an integral part of the culture of many societies of the world. Many herbs and herbal recipes.

AIM AND OBJECTIVES

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs,

which are utilized as therapeutic agents. Such a fact is cause for concern, because of the number of patients in hospitals who have suppressed immunity, and due to new bacterial strains, which are multi-resistant. Consequently, new infections can occur in hospitals resulting in high mortality. From 1980 to 1990, Montelli and Levy documented a high incidence of resistant microorganisms in clinical microbiology in Brazil. This fact has also been verified in other clinics around all over world. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient. For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. The use of plant compounds for pharmaceutical purposes has gradually increased in Brazil. According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant.

PLANT PROFILE**HISTORY OF NYCTANTES ARBOR-TRISTIS:**

Nyctanthes arbortristis is a species of Nyctanthes native to South Asia and Southeast Asia. [2][3][4][5] It is commonly known as night-blooming jasmine, tree of sadness, tree of sorrow, hengrabubar, coral jasmine [6] and in Singapore serigading. [7] Despite its common name, the species is not a "true jasmine" and not of the genus Jasminum.

Synonyms: Parijat, Night Jasmine, Herringer, Shiuli, Night flowering Jasmine Tree of sadness, hangar bulbar.

Biological sources: It is derived from the flowers of the plant Nyctanthes arbortristis belongs to Magnoliopsida or Dicotyledonae. The plant is a

member of the Oleaceae family

BIOLOGICAL CLASSIFICATION:



Kingdom: Plantae Class: Eudicots Division: Angiosperm Order: Lamiales Genus: Nyctanthes Species: arbor-tristis

Chemical constituents: Nyctanthoside, Beta-sitosterol, Calceolarias, Arbor tristist side A, Nyctanthic acid, crocitecin, Betulinic acid, Ursolic acid, 6-beta hydroxyl.

MEDICINAL PROPERTIES OF NYCTANTHES ARBORTRISTIS:

➤ Antibacterial and Antifungal Neem possesses strong antibacterial and antifungal properties. It is effective against a wide range of bacteria and fungi, including those responsible for skin infections, such as acne, eczema, and ringworm.

➤ Antiparasitic: Neem extracts have been found to be effective against certain parasites, including intestinal worms and malaria-causing parasites. It may help in the treatment and prevention of parasitic infections.

➤ Antidiabetic: Nyctanthes arbor-tristis has been traditionally used to help manage diabetes. It may help lower blood sugar levels by improving insulin sensitivity and reducing glucose absorption in the Intestine.

➤ Anti-pyretic: (from anti- 'against' and pyretic 'feverish') is a substance that reduces fever. [1] Antipyretics cause the hypothalamus to override a prostaglandin-induced increase in temperature. The body then works to lower the temperature, which results in a reduction in fever.

➤ Anti-inflammatory activity: nyctanthes Arbor - tristis Bakr et al. prepared 1-phneyl pyrazolol [3,4-d] pyrimidine derivatives which were found to be anti -inflammatory agents, COX inhibitors, and ulcerogenic agents.

HISTORY OF SALVADORA PERSICA:

Meswak (also referred to as Miswak) is a fluoride-free toothpaste brand that was launched in India by Balsara Hygiene in 1998.[1] The toothpaste is marketed as a herbal toothpaste[2] as it is made from extracts of the *Salvadora persica* plant.[3] The teeth cleaning Twinge of the plant is reputed to have been used over 7,000 years ago.[4] Synonym: Arak Galenia asiatica, Meswak, Peelu, pilu, Mustard free, Salvadorm indica (or) Natural Tooth brush Tree. Biological sources; *Salvadora persica* is a Tree that belongs to the Salvadorecea family.

BIOLOGICAL CLASSIFICATION:



Scientific Classification:

Kingdom: Plantae Division; Magnoliophyta Class; Mag noliopsida Order: Brassicales Family: Salvadoraceae Genus: *Salvadora* Species; *Persica oleoides*

Chemical Constituents: Favooqietal al isolated benzyl-iso-thiocyanate from *Persica* root and also found saponins along with tannins, silica, a small amount of resin, trimethylamine and a fairly large amounts of alkaloids.

MEDICINAL PROPERTIES OF SALVADORA PERSICA:

Antimicrobial: *Salvadora persica* exhibits strong antimicrobial activity against a wide range of bacteria, fungi, and viruses. It can be used topically to treat minor cuts, wounds, and infections.

Antioxidant: meswak leaves contain antioxidants such as flavonoids and phenolic compounds, which help neutralize free radicals and protect cells from oxidative damage.

Anti-inflammatory: *Salvadora persica* has anti-inflammatory properties, which can help reduce inflammation and relieve pain associated with conditions such as arthritis, muscle aches, and joint pain.

Anti-Fungal: *Salvadora persica* had Antifungal

medicines treat fungal infections. Fungus in the soil, air and on your skin can cause yeast infections, ringworm, and nail and skin infections. Breathing in fungal spores can lead to respiratory illnesses. People who have weak immune systems are more prone to fungal infections that require antifungal medicine 22

Antioxidant: *Salvadora persica* contain antioxidants such as flavonoids and phenolic compounds, which help neutralize free radicals and protect cells from oxidative damage from headaches, sore throat, and muscle pain when applied topically or inhaled.

HISTORY OF MENTHA PIPERITA:

Peppermint (*Mentha - piperita*) is a hybrid species of mint, a cross between water mint and spearmint.

[1] Indigenous to Europe and the Middle East,[2] the plant is now widely spread and cultivated in many regions of the world.[3] It is occasionally found in the wild with its parent species.[3][4] Synonyms *Mentha* oil, *oleum Mentha piperita*, *colpermin*, brandy mint, lamb mint.

Biological sources: Freshly collected flowering tops of plant *Mentha piperita*, *mentha arvensis* var *piperascens* (Family - Labiatae) are steam distilled to obtain mentha oil.

BIOLOGICAL CLASSIFICATION:



Kingdom: plantae Division: Archaeplastida Class: Magnoliopsida Order: Lamiales
Family: Lamiaceae Genus: *Mentha*
Species: *Mentha piperita*

CHEMICAL CONSTITUENTS:

Volatile oil is the principle component of peppermint, whose yield is 0.71.5%. The volatile oil consists of carvo (1%), menthol (30-55%), limonene (15%-5%), mentho-furan (1-9%) which is responsible for resinification of o iso-menthone (1.5-10%), methyl acetate (2.8%-10%), cineole (3.5- 14%), pulegone (4%) and menthone (14-32%).

Flavonoids, rutin, carotene, tannins (6-12%), betaine and choline are also present.

MEDICINAL PROPERTIES OF MENTHA PIPERITA

Antimicrobial: *Mentha piperita* exhibits strong antimicrobial activity against a wide range of bacteria, fungi, and viruses. It can be used topically to treat minor cuts, wounds, and infections.

Antioxidant: *Mentha piperita* leaves contain antioxidants such as flavonoids and phenolic compounds, which help neutralize free radicals and protect cells from oxidative damage.

Anti-inflammatory: *Mentha piperita* has anti-inflammatory properties, which can help reduce inflammation and relieve pain associated with conditions such as arthritis, muscle aches, and joint pain.

Anti-Fungal: *Mentha piperita* had Antifungal medicines treat fungal infections. Fungus in the soil, air and on your skin can cause yeast infections, ringworm, and nail and skin infections. Breathing in fungal spores can lead to respiratory illnesses. People who have weak immune systems are more prone to fungal infections that require antifungal medicine.

Anti-oxidant: *Mantha piperita* contain anti-oxidants such as flavonoids and phenolic compounds, which helps neutralize free radicals and protect cells from oxidative damage from head ache, sore throat and muscle pain when applied topically or inhaled.

MATERIALS AND METHODS:

INSTRUMENTS; Autoclave, incubator, hot air oven, Weighing balance, micropipette, inoculation loop, laminar air flow.

GLASS WARE; Petri plates, volumetric flask, test tube, beakers, conical flask, glass rod.

COLLECTION AND AUTHENTICATION OF PLANT MATERIAL:

The plant material *Nyctanthes arbortristis*, *Mentha piperita*, *Salvadora persica* were collected locally in the town of Nuzvid

PREPARATION OF ETHANOLIC EXTRACT

Method: The Ethanolic extract of the plant was prepared using reflex condensation process. The dried leaves coarse powder weighed about 200g and placed in a 500 ml round bottom flask with 200ml of ethanol and its refluxed for 8 hours at 40°C. Then suspension was filtered through a fine muslin cloth. The solvent was evaporated by heating until ¾ is reduced. The remaining solvent is evaporated under room temperature. A semisolid residue was obtained.

PHYTOCHEMICAL SCREENING TESTS

TEST FOR GLYCOSIDES: A portion of the extract was hydrolyzed with HCl and the hydrolysate was subjected to Legal's and Borntrager's test to detect

the Presence of different Glycosides.

LEGAL'S TEST: To the extract, 1ml of Pyridine and few drops of Sodium nitroprusside were added and it was made alkaline with NaOH. Appearance of pink to red colour shows the Presence of glycosides.

BORTRAGER'S TEST: Extract was treated with Chloroform and then the chloroform layer was separated. To this equal quantity of dilute Ammonia solution was added. Ammonia layer acquires pink colour showing the Presence of Glycosides.

TEST FOR SAPONINS

FROTH TEST: Place 2ml of extract in water in a test tube. Shake well, stable froth (Foam) is formed. Hence saponins

TEST FOR ALKALOIDS

DRAGENDORFF'S REAGENT: Drug extract when treated with Potassium Bismuth Iodide Solution gives reddish brown ppt. Hence Alkaloids are present.

MAYER'S REAGENT: drug extract when treated with Potassium Mercuric Iodide solution gives cream color precipitate. Hence alkaloids are Present.

TEST FOR TANNINS: To the extract few ml of Chromic acid was added. No ppt was found. Hence Tannins are present.

TEST FOR FLAVONOIDS

SHINODA TEST: To the extract add few Magnesium turnings and con HCl drop wise. Pink scarlet, crimson red or occasionally green to blue colour appears after few mins. Hence Flavonoids are present.

ZINC HYDROCHLORIDE TEST: To the extract add a mixture of Zn dust and con HCl. Gives red colour after few min. Hence Flavonoids are present.

TEST FOR MUCILAGE: To the extract Ruthenium red solution is added, pink color is obtained. Hence mucilage is present.

TEST FOR CARBOHYDRATES

MOLISH TEST: To the extract add few drops of alcoholic α -naphthol, then add few drops of con. Sulfuric acid through sides of the test tube, violet colour ring is appeared at the junction. Hence Carbohydrates are present.

TEST FOR PROTEINS

XANTHOPROTEIC TEST: To 5ml of extract add 1ml of con Nitric acid and boil. Yellow ppt was obtained. After cooling add 40% NaOH solution. Orange colour was obtained. Hence proteins are present.

TEST FOR PHYTOSTEROLS:

SALKOWSKI TEST: To the extract add few drops

of con sulfuric acid, re colour at the lower layer indicate the Presence of sterol and yellow colour Presence indicate. Hence Phytosterols are present.

ESTIMATION OF ANTIMICROBIAL ACTIVITY

MEDIA

Nutrient agar media.

PREPARATION OF NUTRIENT AGAR MEDIA:

Beef Extract: 0.75%

Peptone: 1.25%

Agar: 5.0%

Sodium Chloride: 0.125% Distilled water: Up to 250 ml. **PROCEDURE**

1. Prepare 100 ml of Nutrient Agar. To weigh out beef extract first tongue depressor, dip into the beef extract and weigh.
2. Adjust the amount of beef extract until the correct amount is obtained.
3. Be sure to be careful not to get beef extract on to the balance.
4. You need to weigh out enough beef extract to get a 0.3% solution.
5. Place the tongue depressor into the flask, beef extract side down.
6. Take a weigh boat and weigh out enough peptone and that to the flask.
7. Add 100ml of distill water and swirl to dissolve the peptone and beef extract. Check the pH, it should be 7.0.
8. Take a weigh boat and weigh out enough agar and add that to the flask.
9. With a Bunsen burner, tripod, asbestos wire-gauze, heats the medium to boiling to dissolve the agar.

PRECAUTIONS:

- Keep the rotating flask to prevent the agar from cooking onto the bottom of the flask.
- Watch out boiling agar can froth and boil out all over the lab bench. As soon as it begins to boil, take it off the heat and put it on to the bench.
- Allow it to cool for a few minutes, While the agar is still warm, but not hot, pipette 3 ml each into 413x100 mm.
- screw cap culture tubes. Label the flask and write your tubes with your name.
- After preparation of your medium, the instructor will take you to the autoclave. Place your media in the autoclave with those of the rest of the class.
- After discussion of the parts of the autoclave, autoclave the medium for 20 minutes.

Estimation of TBC

- Take 13 Petri plates in which 4 Petri plates was act as test for each extract and 1 petri plate acts

as blank

- Add 1ml of test sample media into the petri plate (which was prepared for total bacteria

count) and then it was incubated at 22°C for 5 days. After incubation the zone of inhibition was studied in the petri plates.

Table :1

SI no	Media Type	Technique	Sample included	Pathogen	No of pates		Temperature (°C)	Incubation period
					Blank	Test		
1	NAM	Cup-plate	1 ml each	E. coli	1	4	22	5 days
2	NAM	Cup-plate	1ml each	Bacillus	1	4	22	5 days
3	NAM	Cup-plate	1ml each	Salmonella	1	4	22	5 days
4	NAM	Cup-plate	1ml each	Candida	1	4	32	5 days
5	NAM	Cup-plate	1ml each	Aspergillus	1	4	32	5 days

RESULTS AND DISCUSSION:

8.1. PERCENTAGE YIELD OF THE EXTRACT:

Table:2

SL.NO	Name of The Plant	Percentage Yield (%)
1	Nyctanthes arbortristis	12.3 %
2	Salvadora persica	9.8 %
3	Mentha piperita	10.07 %

8.2. PHYTOCHEMICAL SCREENING:

Table: 3

SI. No	Name of the plant	Alk	Carb	Gly	Tan	Phytos	Flav	Sapo	Pro	Muci
1	Nyctanthes arbortristis	+	+	-	+	+	+	+	-	+
2	Salvadora persica	-	+	-	+	+	+	+	+	+
3	Mentha piperita	+	+	-	+	-	+	+	-	-

The above table indicates the presence (+) or absence (-) of phytochemicals in ethanolic extract (Alk: Alkaloids, Carb: Carbohydrates, Gly:Glycosides, Tan: Tannins, Phytos: Phytosterol, Flav:Flavanoids , Sapo:Saponins , Pro: Proteins , Muci: Mucilages)

Table:4

SL.NO	Name of extract	Zone of inhibition (mm)
1	Nyctanthes arbortristis	28.05
2	Salvadora persica	26.50
3	Mentha piperita	29.08
4	Herbal formulation	27.02

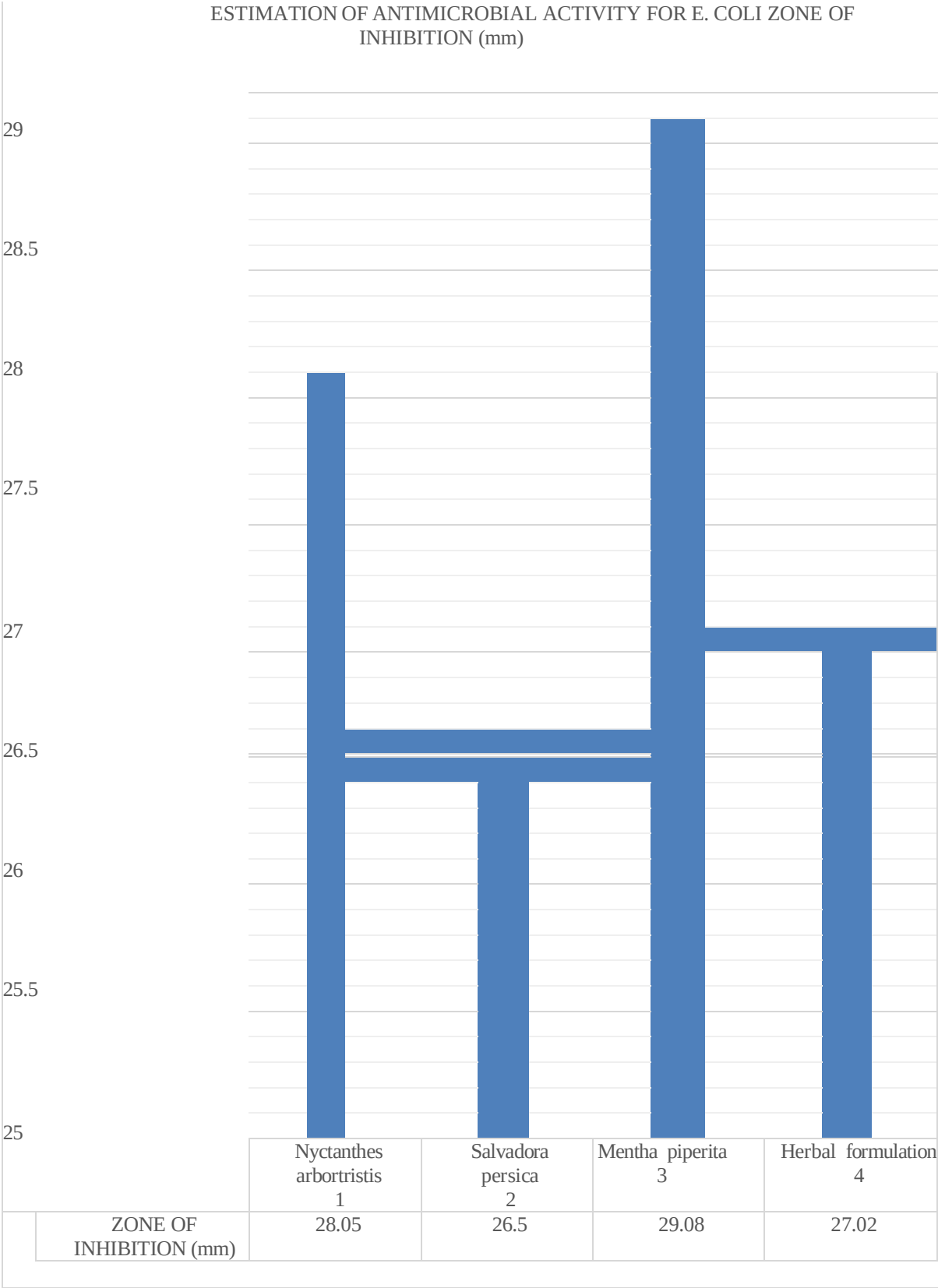
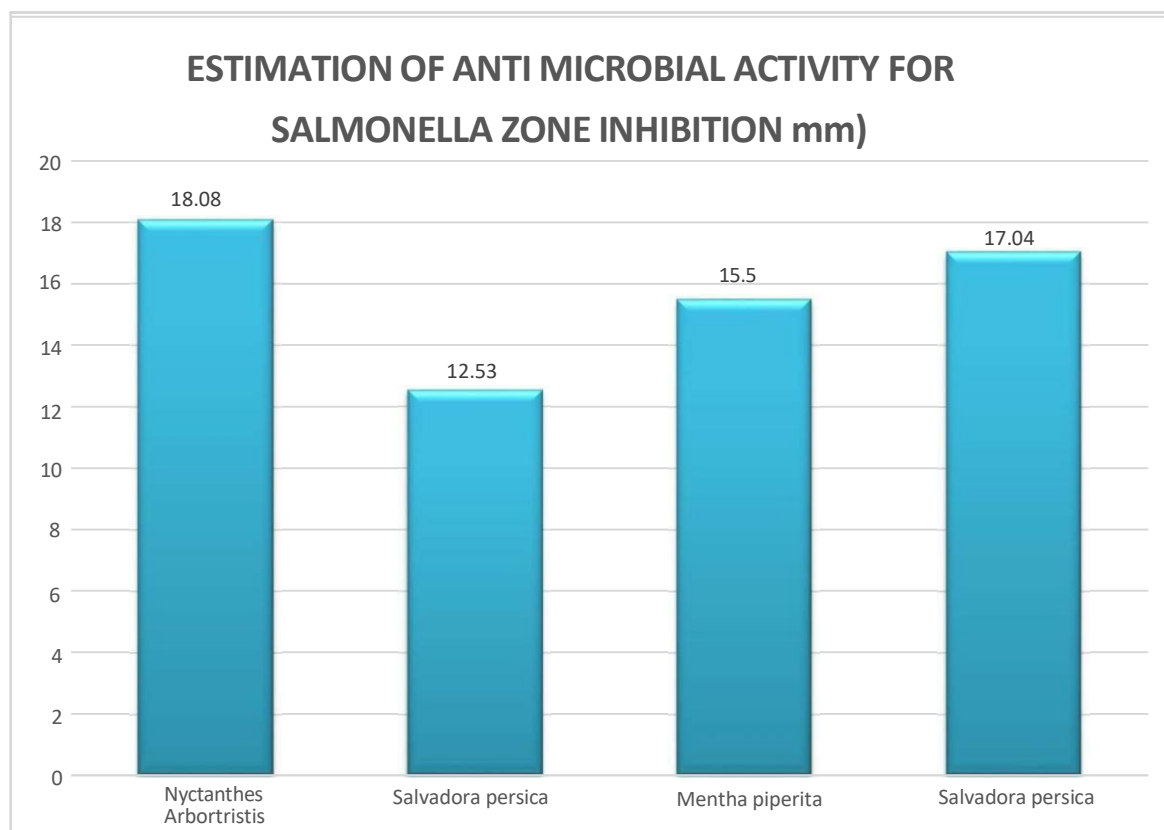


Table:5

Estimation of antimicrobial activity for salmonella		
s.no	Name of extract	Zone of inhibition (mm)
1	<i>Nyctanthes arbortristis</i>	18.08
2	<i>Salvadora persica</i>	12.53
3	<i>Mentha piperita</i>	15.50
4	Herbal formulation	17.04





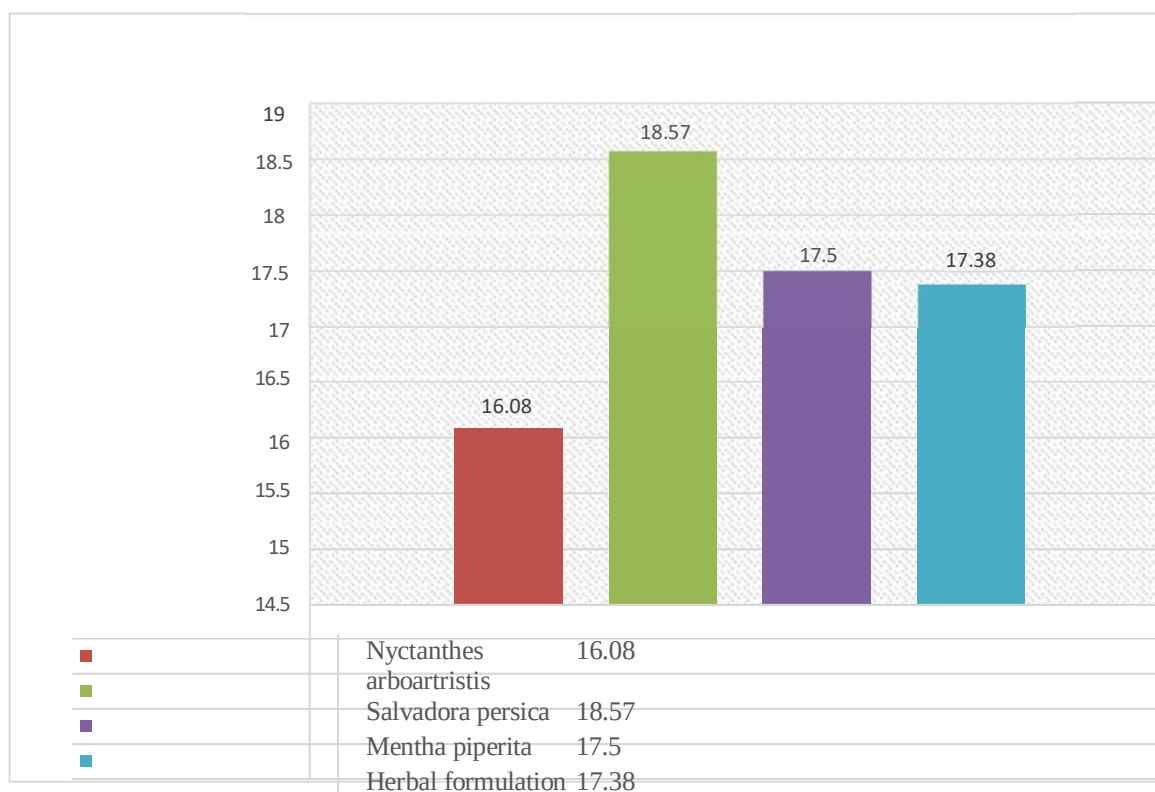
Observing of zone of inhibition by colony counter

ESTIMATION OF ANTI FUNGAL ACTIVITY**PREPARATION OF POTATO DEXTROSE AGAR (PDA);**

1. To prepare potato infusion, boil 50 g sliced, unpeeled potatoes in 250 ml distilled water for 30 min.
2. Filter through cheesecloth, saving effluent, which is potato infusion (or use commercial dehydrated form). Mix with Dextrose, Agar and Water and boil to dissolve
3. Autoclave 15 min at 121°C.
4. Dispense 20-25 ml portions into sterile 15 × 100 mm petri dishes. Final pH, 5.6 ± 0.2.

S.NO	INGREDIENTS	QUANTITIES TAKEN
1	potatoes	50gm
2	Dextrose	5gm
3	Agar	5gm
4	Distilled Water	250ml

**ESTIMATION OF ANTIMICROBIAL ACTIVITY
FOR FUNGI (mm)**

**PRECAUTIONS:**

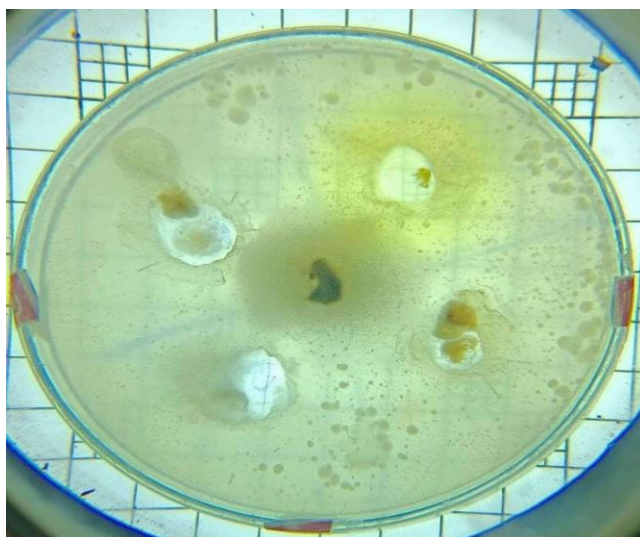
- This product is for laboratory use only and should only be used by qualified, trained laboratory personnel.
- Personnel should always use proper aseptic technique and observe all biohazardous precautions.
- All microbiological culture should be presumed to be infectious.
- Avoid ingestion, inhalation, or contact with skin and mucous membranes.
- If contact occurs, flush the area with clean water.

INNOCULATION OF FUNGUS CULTURES:

- Cultivate fungi isolates on potato dextrose agar [27°C] for 1 week.
- Inoculate a small piece of mycelium in center of new PDA plate
- Culture fungi at 27 °C to grow to 2cm in diameter [3to5days]
- Place disk preloaded with chemical extract pure compound around fungal colony.

- Keep at 27 °C for 4 to 7 days [depending on strain.
- Measure distance between center and edge of fungal colony daily. Terminate when fungal colony grows over control. **Table:6**

Estimation of antimicrobial activity for fungi		
s.no	Name of extract	Zone of inhibition (mm)
1	<i>Nyctanthes arbortristis</i>	16.08
2	<i>Salvadora persica</i>	18.57
3	<i>Mentha piperita</i>	17.50
4	Herbal formulation	17.38



DISCUSSION:

From the table -1 we have come to know the percentage yield of the ethanolic herbal extract were obtained in which the *Azadiracta indica* is having highest yield is about 13.1 % and the lowest is *Tridax procumens* is about 9.8%

The percentage yield of the herbal extract were arranged in descending order :

From the table -3 shows all the ethanolic herbal extracts contain

(Alk: Alkaloids, Carb: Carbohydrates, Gly: Glycosides, Tan: Tannins, Phtos: Phytosterol, Flav: Flavonoids , Sapo: Saponins , Pro: Proteins, Muci: Mucilages)

Nyctanthes arbortristis have Alkaloids, Carbohydrates, Tannins, Phytosterol, Flavonoids, Saponins, Mucilages but absence of Glycosides and Proteins

Salvadora persica, Carbohydrates, Tannins, Phytosterol, Flavonoids, Saponins, Proteins, Mucilages but absence of Glycosides and Alkaloids.

Mentha piperita Carbohydrates, Tannins, Phytosterol, Flavonoids, Saponins, Proteins, Mucilages but absence of Glycosides and Alkaloids. In which *Mentha piperita* have a higher antibacterial activity for *E. coli*.

From the table no:5 the antibacterial activity *Nyctanthes arbortristis*, *Herbal formulation* for salmonella. where as all the two extracts have a significant result..

In which *Nyctanthes arbortristis*, have a higher antibacterial activity for salmonella

From the table no:6 the anti-fungal activity, Mucilage but absence of Glycosides and Proteins

Salvadora persica, *Mentha piperita* was seen for bacillus. where as all the two extract have a significant result.

CONCLUSION:

From this study we can conclude that selected medicinal plants under this study had antibacterial activity against common bacterial species and had relatively broad-spectrum antibacterial activity (inhibited 6 out of 10 bacterial species tested). Plants are important source of potentially useful structures for the development of new drugs. the results of the present investigation prove that the plant extracts have antibiotic activity which may be useful for further investigations. Further extensive study to investigate their activity and inhibition spectrum will give us more knowledge as well as clinical utilization of these plant extracts. The results of the present

investigation clearly indicate that the antibacterial activity vary with the species of the plant and plant's parts. As the investigation goes on the anti-fungal activity has been detected by conducting invitro studies on fungi which shows a massive result against by the herbal formulation that can cure fungal diseases which can be related to human beings.

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