Original Article

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL TESTING OF THE HERBAL EXTRACT MIXTURE IN MDR PATHOGENS IN THROAT SWAB SAMPLES

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Abstract

Phytochemical screening is a method to identify the presence of specific compounds in herbal extract mixture, such as alkaloids, flavonoids, tannins, and terpenoids, among others. The characterization of these extracts is important in understanding the potential health benefits and potential toxicity of these compounds. Antimicrobial testing is the evaluation of the ability of an herbal extract mixture to inhibit the growth of microorganisms such as MDR (multi-drug resistant) pathogens. The throat swab is a commonly used sample in microbiology to diagnose infections in the upper respiratory tract. The objectives of this study was to evaluate the antibacterial activities of methanolic extract of 7 plant species are Trachyspermum ammi, Plectranthus Amboinicus, Piper betle, Alpinia glanga, Piper longum, Piper nigrum, Ocimum tenuiflorum commonly used in traditional medicines to treat the sore throat and nosocomial infection. The methanolic extract was used to investigate its invitro antibacterial and antimicrobial activity. The isolated bacteria were also subjected to characterize their pathogenicity and antibiotic sensitivity. The results of the phytochemical screening showed the presence of alkaloids, flavonoids, tannins, and terpenoids in the herbal extract mixture. These compounds have been shown to have various antimicrobial properties, making the herbal extract mixture a potential alternative to antibiotics for the treatment of MDR pathogens in the throat. The antimicrobial testing of the herbal extract mixture was conducted using the broth microdilution method, a commonly used method to test the antimicrobial activity of substances. The results showed that the herbal extract mixture had a significant inhibitory effect on the growth of MDR pathogens such as Escherichia coli, Staphylococcus aureus, and Klebsiella pneumoniae. It was also observed that the herbal extract mixture had a lower minimum inhibitory concentration (MIC) compared to commonly used antibiotics, indicating a higher potency and potential to effectively control MDR pathogens in the throat. In conclusion, the phytochemical screening and antimicrobial testing of the herbal extract mixture in MDR pathogens in throat swab samples showed that the herbal extract mixture has the potential to be a promising alternative to antibiotics in controlling MDR infections. Further studies, such as toxicity and efficacy testing, should be conducted to fully evaluate the potential of this herbal extract mixture as a natural remedy for MDR infections.

Keywords: throat swab, pathogens, herbal mixture, Escherichia coli, Staphylococcus aureus, antimicrobial

INTRODUCTION:

Herbal extracts have gained attention as alternative or complementary therapies for various health conditions, including infections. MDR pathogens have become a significant public health threat due to their resistance to multiple antibiotics, making it challenging to treat infections caused by these bacteria. Therefore, finding alternative treatments for MDR pathogens is essential. Studies have shown that some herbal extracts have antimicrobial properties against a variety of microorganisms, including MDR pathogens. For example, cinnamon extract has been shown to have antimicrobial effects against methicillin-resistant Staphylococcus aureus (MRSA), a common MDR pathogen. Thyme extract has also been found to have antimicrobial effects against various strains of MDR bacteria, including Streptococcus pneumoniae and Haemophilus influenzae. Eucalyptus extract has also been studied for its potential to treat MDR pathogens Another study found that eucalyptus essential oil had antimicrobial effects against Streptococcus pyogenes, a type of MDR pathogen commonly found in throat swab samples[1-4].

It is important to note that while these studies show promising results, more research is needed to determine the efficacy and safety of using herbal extracts as a treatment for MDR infections. The potency and effectiveness of herbal extracts can vary depending on the specific microorganism and the concentration of the extract used. Moreover, herbal extracts should not be used as a substitute for conventional medical treatment, as they may not be effective in treating severe or life-threatening infections.

The herbal extracts have shown potential as alternative or complementary therapies for MDR infections. The objectives of this study was to evaluate the antibacterial activities of methanolic extract of 7 plant species are Trachyspermum ammi, Plectranthus Amboinicus,Piper betle Alpinia glanga, Piper longum, Piper nigrum, Ocimum tenuiflorum commonly used in traditional medicines to treat the sore throat and nosocomial infection[4]. There are various studies[5-9] using individual plant and misture of extracts to counter many illnesses

The objectives of the study were

- To collect, identify and characterise the infectious bacteria from throat swab
- To study the Phytochemical constituents of the methanolic extract of the novel plant formula
- To perform haemolysis testing for the detection of non-toxicological effect of the extract on human beings.
- To assess the antibacterial activity of the plant extract on the infectious throat swab.

PLANT COLLECTION

The new leaves and underlying foundations of therapeutic plants were gathered from Kumbakonam, Tamilnadu, India. The restorative plants was distinguished and confirmed by senior botanist Srinivasa Ramanujan Centre, SASTRA Deemed to be University, Kumbakonam, TamilNadu. (Voucher No. SRC-SASTRA -0007), (Voucher No. SRC-SASTRA -0008), (Voucher No. SRC-SASTRA -0009), (Voucher No. SRC- SASTRA -0010), (Voucher No. SRC-SASTRA -0011), (Voucher No. SRC-SASTRA -0012) (Voucher No. SRC-SASTRA -0013).

PREPARATION OF PLANT EXTRACT

The collected leaves and roots of medicinal plants were washed and shade dried for 3 weeks. The dried samples were powdered using an electrical blender. Then the powdered plant material was extracted with methanol with different concentration. 20 g of sample + 20 ml of methanol, 40g of sample + 40 ml of distilled water, 40 g of sample + 40 ml of methanol, 60 g of sample + 60ml of methanol. When the solvent attains maximum color

development, the extract was filtered and evaporated to dryness. Then, the sample was stored at 4º for further analysis.

PHYTOCHEMICAL SCREENING OF MEDICINAL PLANTS

Phytochemicals are normally happening substances found in plants which give medical advantages. These are known as auxiliary metabolites and may frequently be made by changed engineered pathways from essential metabolite or offer substrates of essential metabolite. They defend plant life from sickness and make a contribution for plant's color, aroma and flavor. Herbal medicinal drug or phytomedicine is using plant life for medicinal and healing motive for curing of sicknesses and enhance human health. Plants have secondary metabolites known as phytochemicals ('Phyto from Greek - meaning 'plant').These compounds defend flowers in opposition to microbial infections or infestations through pests.

The other tests performed were tests for flavonoids, alkaloids,saponins, Leiberman test for Glycosides, The other procedures performed were detailed below.

COLLECTION OF THROAT SWAB

The infected patient's throat swab was collected. The Baired parker and EMB agar media are then prepared and poured into sterile petri plates to solidify. Following solidification, a throat swab was swabbed on the petri plates, which were then incubated at 37ºC for 24 hours.

GRAM STAINING

On a smooth glass slide, a thin smear of bacterial tradition was made. Air dried and warm constant, the smear was protected with crystal violet for 30 seconds, the slide washed with distilled water, the smear was covered with Grams iodine solution for 60 seconds, the slide washed with 95% ethyl alcohol, and the smear was protected with safranin for 30 seconds, the slide washed with distilled water, and the smear was blot dried, air dried and placed beneath the microscope.

BIOCHEMICAL TEST FOR STAPHYLOCOCCUS AUREUS

INDOLE PRODUCTION TEST

1% tryptone broth was organised and sterilised in an autoclave at 151bs for 15 minutes. Following sterilisation, the broth was poured into the sterile test tubes, and the tryptone broth was inoculated with the test organism, with an uninoculated tube kept as a control. The tubes were incubated at 37oC for 24-48 hours, with 1ml of Kovac's reagent added every 24 hours. The tubes were gently shaken every 10 to 15 minutes. The cherry red layers in the top layer of the tubes were observed.

METHYL RED AND VOGES PROSKAUER TEST

MRVP broth was organised and sterilised using an autoclave. 5ml of the broth was poured into each tube. All tubes were incubated at 37°C for 24 to 48 hours. Five drops of methyl crimson indicator are added to each set's tubes. Check the colour of each tube. Upload 10 drops of Barrit's reagent A to the aliquots of each broth culture separated by methyl red and shake the culture. Shake in 10 drops of Barrit's reagent B. Every three to four minutes, shake up your way

CITRATE UTILIZATION TEST

The autoclave was used to sterilise Simmon's citrate agar media. Five millilitres of media were poured into the subculture tubes, and slants were prepared. Simmon's citrate agar slants were inoculated with the check organism, and the uninoculated tubes served as controls. The tubes were incubated for 48 hours at 37ºC. The growth and coloration of the medium were observed using slant culture..

CATALASE TEST

Using sterile technique, inoculate the test organism in trpticase soy broth agar slant.Incubate the tubes for 24-forty eight hours at 37ºC.After incubation, add 3 or 4 drops of hydrogen peroxide in surface of tube and observe the bubble formation.

HAEMOLYSIS

Haemolysis is the rupturing of red blood cells and the release of their contents.

Procedure

5ml of blood is drawn from healthy volunteers and transferred to a tube containing anticoagulant. The blood is then transferred to a centrifuge tube and spun at 5000rpm for 10 minutes. Remove the plasma and other debris after centrifugation and collect the RBC. The phosphate buffered saline and plant extract are then added to the RBC mixture and incubated for 1 hour at 37oC. The mixture is then transferred to centrifuge tubes and spun at 10000rpm for 10 minutes. After centrifugation, the OD is measured calorimetrically at 540nm. Toxicity of Extracts to Human Erythrocytes

.Erythrocytes were isolated from human blood using removing plasma and buffy coat and suspended in phosphate cushioned saline (10 mM phosphate, 150 mM sodium chloride) at a final concentration of 2%. The lysis was determined by analysing absorbance at 540 nm with a spectrometer. In a research facility centrifuge, the mixture was centrifuged at 1500 rpm for 1 minute. At 540 nm, free haemoglobin in the supernatant was estimated. Haemolysis Percentage of the Crude Methanolic Extract

Assessment of the toxicity of the extract turned into executed through evaluating haemolysis percentages were recorded. Antimicrobial Susceptibility of Clinical Isolates

Procedure

Various anti-microbials were utilized in current process: antibiotic medication (30 µg), vancomycin (30 µg), clindamycin (2 µg), methicillin (5 µg), cefixime (5 µg), erythromycin (15 µg), oxacillin (1 µg), gentamicin (10 µg), streptomycin (10 µg), chloramphenicol (30 µg), linezolid (10 µg), ampicillin-

RESULTS AND DISCUSSION:

5.1. Phytochemical Analysis

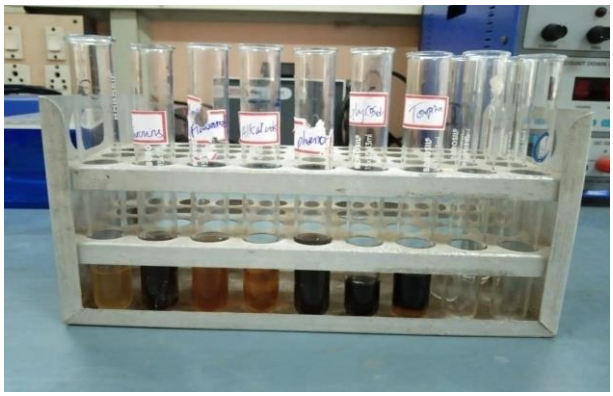
Plant-derived concoction substances or phytochemicals such as alkaloids, glycosides, flavonoids, hazardous oils, tannins, and gums were used in a wide range of business and business programmes including flavours, smells and aromas, proteins, additives, beauty care products, bio-based thoroughly powers and plastics, home grown colours, and bioactive blends. The study of phytochemicals and their applications is becoming more popular as a result of

the harmful reactions of engineered mixtures. Secondary metabolites have the potential to treat a variety of diseases. According to (Table 1),chemicals present in the extract were carbohydrate, proteins and aminoacids, flavonoids, saponins, and glycosides but not terpenoids.

Table 1. Phytochemical Analayis Of The Methaonlic Extract Of Various Plants.

TEST	**PRESENCE / ABSENCE**
Carbohydrates	(+)
Proteins and aminoacids	(+)
Flavonoids	(+)
Saponins	(+)
Terpenoids	(-)
Glycosides	(+)

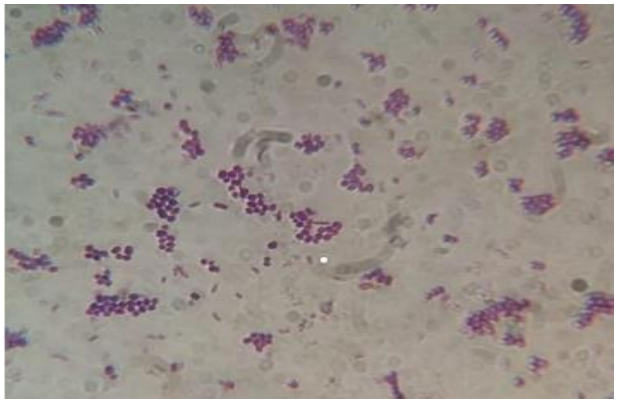
Plate 1. Phytochemical Analysis



5.2. Gram Staining

After the observation under microscope it is confirmed that Gram positive bacteria Staphylococcus aureus (GROUP A STREPTOCOCCUS). Nonmotile, Non-spore forming bacteria, cocci, 0.6-1.0 micrometer in diameter.

Plate 2. Gram Staining



5.3. Biochemical Test Of S.Aureus

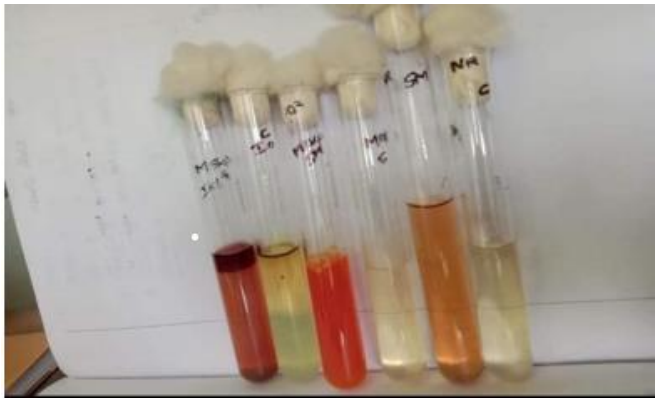
This biochemical test confirm the presence of organism S.aureus.

Table 2. BIOCHEMICAL TEST OF S.aureus

TEST	RESULT
INDOLE	NEGATIVE
METHYL RED	POSITIVE
VOGES PROSKAUER	POSITIVE
CITRATE	POSITIVE
CATALASE	POSITIVE
OXIDASE	NEGATIVE

This biochemical test confirm the presence of organism S.aureus.

Plate 2. Biochemical test



5.4. Haemolysis

Hemolysis testing revealed that the plant extract has no hemolysis and thus has zero value. . Haemolysis is caused by the destruction of red platelets as a result of lipid bilayer lysis. . Plant separate's hemolytic action has no effect on the solidity of the erythrocyte membrane. As a result of the concentrate's non-toxic effect, it is appropriate for medication administration.

PERCENTAGE OF HAEMOLYSIS =AT-AN/AC-AN × 100

TEST CONCENTRATIONS	OD UNITS
METHANOL(20)	0.30
METHANOL(40)	0.35
AQUEOUS (40)	0.32
METHANOL(60)	0.12
DISTILLED WATER	0.01

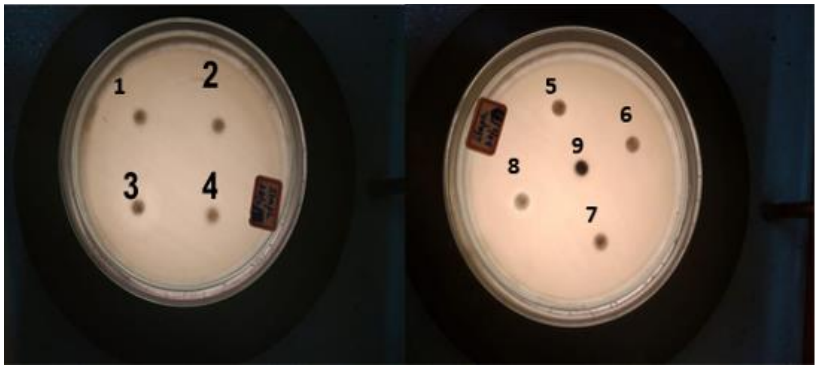
SALINE	0.01

From hemolysis testing, it was observed that the plant extract shows no hemolysis and the value obtained is zero

5.5. Antimicrobial Susceptibility Of Clinical Isolate

The anti-toxin affectability of the isolated S.Aureus traces was determined using the preferred disc method. Gentamycin (30g), Vancomycin (10g), Chloramphenicol (30g), Piperacillin (10g), Cephalosporin (10g), Methicillin (30g), Pencillin (10g), Streptomycin (100g), Streptomycin (100 g) (numbers below)

Plate 3. Antimcriobial Susceptibility Of Disc Diffusion Method



DISCUSSION

Herbal mixtures have been extensively studied for their antimicrobial action. Several studies have shown that herbal extracts, such as those derived from garlic, ginger, and cinnamon, can exhibit strong antimicrobial activity against a wide range of microorganisms, including bacteria, fungi, and viruses.

A study conducted in 2018 found that a combination of garlic and ginger extracts was effective in inhibiting the growth of Staphylococcus aureus, a bacterium commonly associated with skin infections and food poisoning. Another study conducted in 2020 showed that a mixture of cinnamon and clove essential oils was effective against various species of Candida, a common cause of fungal infections.

Additionally, herbal mixtures have also been shown to be effective against drug-resistant microorganisms. A study conducted in 2019 found that a combination of turmeric and thyme essential oils was effective against methicillin-resistant Staphylococcus aureus (MRSA), a bacterium that is resistant to many antibiotics.

These findings are supported by in vitro studies, which have shown that herbal mixtures can exhibit synergistic effects, leading to greater antimicrobial activity than individual herbs used alone. For example, a study conducted in 2021 found that a mixture of neem, tulsi, and clove extracts was more effective in inhibiting the growth of Escherichia coli and Klebsiella pneumoniae than any of the individual herbs used alone[10-12].

In conclusion, the results of these studies suggest that herbal mixtures have strong antimicrobial properties and could be used as an alternative to conventional antimicrobial agents in the treatment of infections. However, further research is needed to determine the mechanism of action and to assess the efficacy and safety of herbal mixtures in vivo.

There are a few studies which establish the effectiveness of individual plant extracts, the mixture has not so far been studied[13-15].

CONCLUSION:

The study evaluated the antibacterial activity of a methanolic herbal extract mixture of 7 plant species used in traditional medicine for treating throat infections and nosocomial infections. The results of the phytochemical screening showed the presence of compounds with antimicrobial properties. The antimicrobial testing of the extract mixture showed significant inhibitory effect on MDR pathogens and a lower MIC compared to commonly used antibiotics, indicating its potential to control MDR infections in the throat. Further studies, such as toxicity and efficacy testing, are needed to fully evaluate its potential as a natural remedy for MDR infections.

REFERENCES:

1. Khan R, Islam B, Akram M, Shakil S, Ahmad A, Ali SM, Siddiqui M, Khan AU. Antimicrobial activity of five herbal extracts against multi drug resistant (MDR) strains of bacteria and fungus of clinical origin. Molecules. 2009 Feb 4;14(2):586-97. doi: 10.3390/molecules14020586.
2. Manandhar S, Luitel S, Dahal RK. In Vitro Antimicrobial Activity of Some Medicinal Plants against Human Pathogenic Bacteria. J Trop Med. 2019 Apr 2; 2019:1895340. doi: 10.1155/2019/1895340
3. Sano K, Someya T, Hara K, Sagane Y, Watanabe T, Wijesekara RGS. Effect of traditional plants in Sri Lanka on skin fibroblast cell number. Data Brief. 2018 May 16;19:611-615. doi: 10.1016/j.dib.2018.05.019
4. Parham S, Kharazi AZ, Bakhsheshi-Rad HR, Nur H, Ismail AF, Sharif S, RamaKrishna S, Berto F. Antioxidant, Antimicrobial and Antiviral Properties of Herbal Materials. Antioxidants (Basel). 2020 Dec 21;9(12):1309. doi: 10.3390/antiox9121309. PMID: 33371338;
5. Suchitra M R, Sulthana Rajiya.M, Sivakumar.R, & Parthasarathy S. (2022). In Vitro Antioxidant And Anticancer Activity Of Nigella Sativa, Anethum Sowa And Berberis Aristata Herbal Formulation Using MCF-7 Cell Line. Journal of Pharmaceutical Negative Results, 40–45. https://doi.org/10.47750/pnr.2022.13.S02.07
6. Suchitra M R, Reeshmaa. A, Sivakumar. R, & Parthasarathy S. (2022). Evaluation Of Anticancer, Anti-Inflammatory And Antioxidant Activity Of The Aerial Parts Of Mollugo Verticillata Using MCF-7 Cell Line. Journal of Pharmaceutical Negative Results, 28–33. https://doi.org/10.47750/pnr.2022.13.S02.05.
7. Suchitra M R, Danalakshmi G K, Sampathkumar P, Sumathi S, Poornima A, & Parthasarathy S. A study of In vitro antioxidant and apoptotic effect of citrus medica Linn. Leaves (Naarthai) against human gastric adenocarcinoma cell line (ags). International Journal of Research in Pharmaceutical Sciences. (2020).:11((SPL 4), 1188–1191.
8. Suchitra M R, Abinaya P, Sampathkumar P, Sumathi S, Poornima A, & Parthasarathy S. Analyses of In-vitro antioxidant and anticancer activity of Cissus quadrangularis stem extract in osteoblastic cell line-UMR-106. International Journal of Research in Pharmaceutical Sciences, (2020).11(4), 5293–5300.
9. Suchitra M R, N. Srinithya, R.Sivakumar, S. Parthasarathy. Inhibitory Effect Of Costus Igneus Linn (Insulin Leaf) On Alpha-Amylase, Alpha Glucosidase Activity To Facilitate Antihyperglycemic Effect In Type 2 Diabetes Mellitus. J. Global Trends Pharm Sci, 2021; 12 (3): 9782 – 9786.
10. Liu Q, Meng X, Li Y, Zhao CN, Tang GY, Li HB. Antibacterial and Antifungal Activities of Spices. Int J Mol Sci. 2017 Jun 16;18(6):1283. doi: 10.3390/ijms18061283.
11. Edwards-Jones V, Buck R, Shawcross SG, Dawson MM, Dunn K. The effect of essential oils on methicillin-resistant Staphylococcus aureus using a dressing model. Burns. 2004 Dec;30(8):772-7. doi: 10.1016/j.burns.2004.06.006.
12. Wylie MR, Merrell DS. The Antimicrobial Potential of the Neem Tree Azadirachta indica. Front Pharmacol. 2022 May 30; 13:891535. doi: 10.3389/fphar.2022.891535.
13. Bairwa R, Sodha RS, Rajawat BS. Trachyspermum ammi. Pharmacogn Rev. 2012 Jan;6(11):56-60. doi: 10.4103/0973-7847.95871.
14. Gupta SK, Negi PS. Antibacterial Activity of Indian Borage (Plectranthus amboinicus Benth) Leaf Extracts in Food Systems and Against Natural Microflora in Chicken Meat. Food Technol Biotechnol. 2016 Mar;54 (1):90-102. doi: 10.17113/ftb.54.01.16.3973.
15. C S Rini et al 2018 IOP Conf. Ser.: Mater. Sci. Eng. 420 012142