



Journal of Innovations in Applied Pharmaceutical Science [JIAPS]

Content available at: www.saap.org.in ISSN: 2455-5177



EVALUATION OF ANTIMICROBIAL ACTIVITY OF A HERBAL MIXTURE

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Article History

Received: 06-10-2023

Revised: 26-10-2023

Accepted: 12-10-2023

Keywords:

O.tenuiflorum –
Ocimum tenuiflorum,
P.nigrum – *Piper*
nigrum-, *P.amboinicus*
– *Plectranthus*
amboinicus-,
Z.officinalae – *Zingiber*
officinalae-, *S.aureus* –
Staphylococcus aureus,
K.pneumoniae –
Klebsiella pneumoniae,
P.aeruginosa –
Pseudomonas
aeruginosa

Abstract

Due to adverse effects of using allopathic medicines, the use and search for pharmaceuticals made from plants has increased recently. A mixture of herbal extracts known as “Chukku Kashaya” is widely used as home remedy in Kerala for respiratory disorders. Additionally, it relieves cold and fever symptoms. The present investigation focused on evaluating the antimicrobial activity of methanolic extracts obtained by soxhlet extraction of leaves of *Ocimum tenuiflorum* and *Plectranthus amboinicus* and fruits of *Piper nigrum* and rhizome of *Zingiber officinalae* and their combination in equal ratios and their activity was determined using agar well diffusion method against *Staphylococcus aureus* (gram positive), *Klebsiella pneumoniae* (gram negative), and *Pseudomonas aeruginosae* (gram negative). Streptomycin was used as positive control. *Zingiberofficinalae* showed maximum zone of inhibition against all the bacteria and herbal mixture showed moderate zone of inhibition. The mixture was more active than *Ocimum tenuiflorum*, *Piper nigrum* and *Plectranthus amboinicus*. *Staphylococcus aureus* was shown to be the most sensitive to all the extracts and herbal mixtures, whereas *Klebsiella pneumoniae* showed the least activity. Though *Z.officinalae* has higher antimicrobial activity, there is limited literature support to prove its activity against fever, cold and other symptoms of respiratory tract infections. *Z.officinalae* is mainly used for GI related disorders. This herbal blend is more efficient because it includes of *O.tenuiflorum*: having antipyretic, analgesic and voice improving activity; *P.amboinicus*: providing relief from cough, asthma, bronchitis and other symptoms of respiratory tract infections; *P. nigrum* which can improve appetite and digestion.




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 <https://doi.org/10.37022/jiaps.v8i3-S.522>

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Introduction

It has been known from ancient times that essential oils from aromatic and medicinal plants possess biological activity, antibacterial, antifungal and antioxidant properties. Due to the growing interest in the use of essential oils in both the food and

the pharmaceutical industries, a systematic study on these plant extracts have become very important.

The interest in the study of medicinal plants as a source of pharmacologically active compounds has increased worldwide. In developing countries like India, plants are the main medicinal sources to treat infectious diseases. The world health organization has estimated that 80% of the people in the world depend upon traditional medicines for the primary health care needs and major part of the therapy involves the use of plant extracts or their active principles. Scientists in many parts of the world have carried out extensive research and have proven the effective use of herbal medicine [1].

Antimicrobial agents may be anti-bacterial, anti-fungal or anti-viral. They all have different modes of action by which they act

to suppress the infection. Infections occur in the respiratory tract more frequently than in any other site. Most of these infections involve the upper respiratory tract. Use of plant extracts and phytochemicals can be of great significance in therapeutic treatment and help to crush the problem of multidrug resistant organism.

The herbal mixture selected for the present study is chukku kashaya, prepared by mixing the crude extracts of leaves of *Ocimum tenuiflorum*, leaves of *Plectranthus amboinicus*, *Zingiber officinalae* and *Piper nigrum* which is used as a home remedy for the treatment of cough, cold and fever. The potential antimicrobial activity was evaluated against *Staphylococcus aureus* (gram positive), *Klebsiella pneumoniae*, and *Pseudomonas aureginosa* (gram negative) by agar well diffusion method.

1. *Ocimum tenuiflorum*-

Ocimum tenuiflorum, commonly known as holy basil, tulsi, is an aromatic perennial plant belonging to the family *Lamiaceae*. It is native to the Indian subcontinent and widespread as a cultivated plant throughout the Southeast Asian tropics.

Tulsi is used in the treatment of various diseases. It is used in case of insect bite, fever, cardiac diseases, gynecological disorders, respiratory problems, skin disorders, etc. Holy basil is used as voice improver and acts as a germicide, antipyretic and bactericide. It is good to prevent malarial and effective painkiller. The leaves have expectorant properties and their expressed juice is employed for variety of purposes in native medicine.

1.1.1. Taxonomical classification [3]

Kingdom : Plantae
 Division : Magnoliophyta
 Class : Magnoliopsida
 Order : Lamiales
 Family : Lamiaceae
 Genus : *Ocimum*
 Species : *tenuiflorum*

2. *Piper nigrum*-

Black pepper (*Piper nigrum*) is a flowering vine in the family piperaceae, cultivated for its fruit which is usually dried and used as a spice and seasoning, known as peppercorn.

Black pepper was believed to cure several illnesses such as constipation, insomnia, oral abscesses, sunburn and tooth aches. It is also used to improve appetite and digestion, as well as to treat stomach ache, heart burn, indigestion, intestinal gas, diarrhea, and cholera.

1.2.1 Taxonomical classification [4]:-

Kingdom : Plantae
 Division : Magnoliophyta
 Class : Magnoliopsida
 Order : piperales
 Family : piperaceae
 Genus : *Piper*
 Species : *nigrum*

3. *Plectranthus amboinicus*-

Plectranthus amboinicus is a semi-succulent perennial plant with ~30-90cm in height and with thick fleshy stem and leaves which belongs to the family *Lamiaceae*. It is native to Southern and Eastern Africa. It is a folkloric medicinal plant used to treat malarial fever, hepatopathy, renal and vesical calculi, cough,

chronic asthma, hiccough, bronchitis, helminthiasis, colic, convulsions and epilepsy.

1.3.1 Taxonomical classification [5]

Kingdom : Plantae
 Division : Magnoliophyta
 Class : Magnoliopsida
 Order : Lamiales
 Family : Lamiaceae
 Genus : *Plectranthus*
 Species : *amboinicus*

4. *Zingiber officinalae*-

Ginger (*Zingiber officinalae*) is a flowering plant belonging to the family Zingiberaceae, and is widely used as a spice or a folk medicine. Ginger is thought to act directly on the gastrointestinal system to reduce nausea. Therefore, it is used to prevent nausea resulting from chemotherapy, motion sickness and surgery. Ginger is also used to treat various types of other GI problems like morning sickness, colic, heart burn, diarrhea, loss of appetite and dyspepsia [7].

1.4.1 Taxonomical classification [6]:-

Kingdom : Plantae
 Division : Manoliophyta
 Class : Magnoliopsida
 Order : Zingiberales
 Family : Zingiberaceae
 Genus : *Zingiber*
 Species : *officinalae*.



Fig.1. *O. tenuiflorum*.



fig.2. *P. nigrum*



Fig.4. *Z. officinalae*



Fig.3. *P. amboinicus*

Materials and methods

Plant Materials

1. *Ocimum tenuiflorum*
2. *Zingiber officinalae*
3. *Plectranthus amboinicus*
4. *Piper nigrum*

Instruments:

1. Autoclave
2. Laminar air flow

Test Organisms

1. *Staphylococcus aureus* (gram positive)
2. *Klebsiella pneumoniae* (gram negative)
3. *Pseudomonas aureus* (gram negative)

Method

Preparation of Plant Extract

Rinsed fresh leaves of *Ocimum tenuiflorum*, *Plectranthus amboinicus*, rhizome of *Zingiber officinalae* and fruits of *Piper nigrum* thoroughly in running tap water, dried at room temperature (22-25°C) for a period of 21 days and pulverised into coarse powder using a mechanical grinder. Extracted approximately 50g of powdered drugs separately using methanol in a Soxhlet apparatus. Distilled and concentrated to yield dry extracts.

Evaluation of Antimicrobial Activity

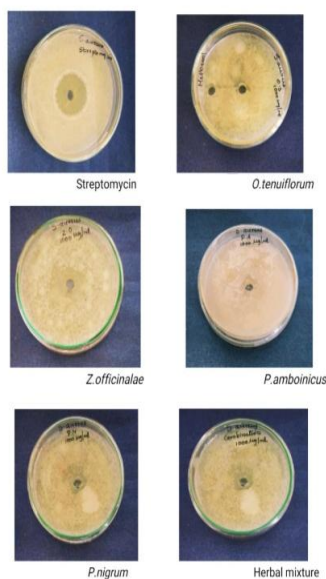
The antimicrobial activity was determined by agar - well diffusion method. 18 petridishes were used for antimicrobial test. Incubated the petridishes in autoclave for 20min. Petri dishes containing nutrient agar medium were seeded with bacterial culture of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Wells of approximately 10mm were bored using cork borer and different concentrations of samples such as 250µg/ml, 500µg/ml, 1000µg/ml were added. Streptomycin (100µg/ml) was used as standard. The wells were filled with 1ml of standard streptomycin and extracts. Labelled and incubated all the plates in an incubator for 24hours at 35° c. The antimicrobial activity was assayed by measuring the diameter of the zone of inhibition formed around the well. Methanol was used as negative control.

Statistical Analysis

The data obtained from different results were analysed by two way analysis of variance (ANNOVA) procedure using the software sigma plot Version 11. A statistically significant difference was considered at p < 0.05.

Result and Discussion

Antimicrobial activity of plant extracts and their combination against *Staphylococcus aureus*:



Antimicrobial activity of plant extract and their combination against *Pseudomonas aeruginosa*:

PLANT EXTRACTS	DIAMETER OF ZONE OF INHIBITION (mm)		
	250 µg/ml	500 µg/ml	1000 µg/ml
<i>O.tenuiflorum</i>	0	0	0
<i>Z.officinalae</i>	15	18	24
<i>P.amboinicus</i>	0	0	16
<i>P.nigrum</i>	0	15	17
Combination	13	16	19

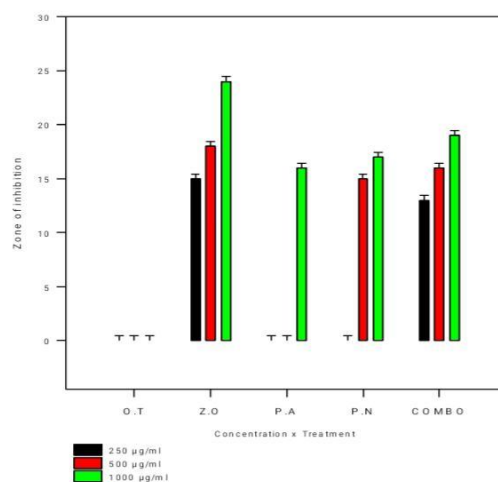
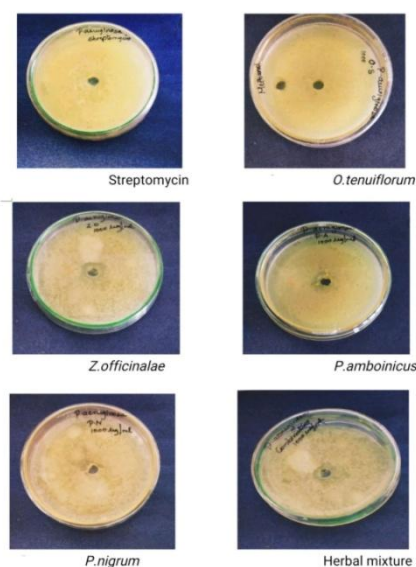


Fig 5: Comparison of zone of inhibition of plant extract against *S.aureus*

Antimicrobial activity of plant extract and their combination against *Pseudomonas aeruginosa*:

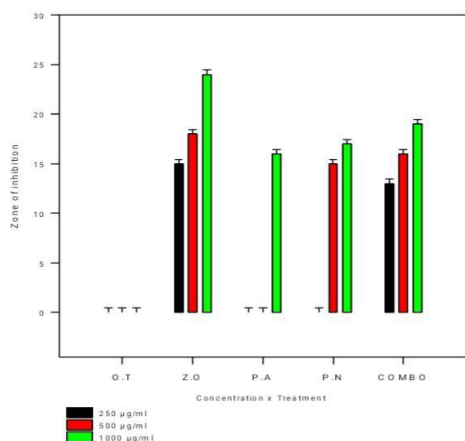


Antimicrobial activity of plant extract and their combination against *Pseudomonas aeruginosa*:

PLANT EXTRACTS	DIAMETER OF ZONE OF INHIBITION (mm)		
	250 µg/ml	500 µg/ml	1000 µg/ml
<i>O.tenuiflorum</i>	0	0	0
<i>Z.officinalae</i>	15	18	24
<i>P.amboinicus</i>	0	0	16
<i>P.nigrum</i>	0	15	17
Combination	13	16	19

Standard Antibiotic : Streptomycin ; Diameter of zone of inhibition- 24mm

Fig6; Comparison of zone of inhibition of plant extracts against *P.aeruginosa*



Antimicrobial activity of plant extracts and their combination against *Klebsiella pneumoniae*:

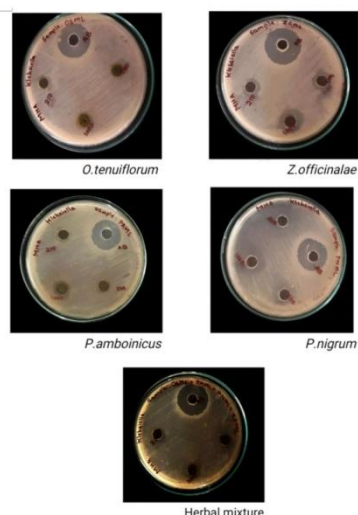
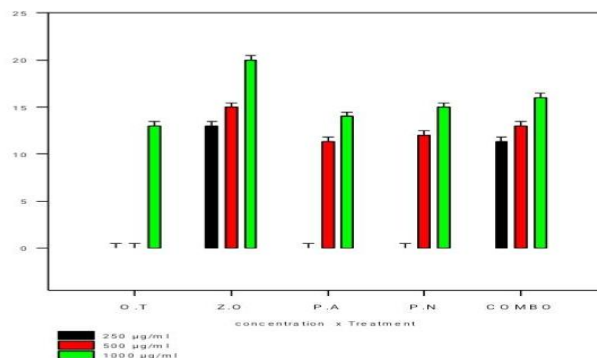


Table no: 3 Antimicrobial activity of plant extracts and their combination against *Klebsiella pneumoniae*:

The present study revealed that the methanolic extract of leaves of *Plectranthus amboinicus*, leaves of *Ocimum tenuiflorum*, fruits of *Pippier nigrum* and rhizomes of *Zingiber officinalae* and their mixture containing equal ratio of these four extracts has antimicrobial activity against the common clinical pathogens like *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. But *O. tenuiflorum* is not active against *P.aeruginosa*. Maximum zone of inhibition was at 1000µg/ml for all the bacterial cultures. This indicates that the antibacterial activity increases as the concentration of plant

PLANT EXTRACTS	DIAMETER OF ZONE OF INHIBITION (mm)		
	250 µg/ml	500 µg/ml	1000 µg/ml
<i>O.tenuiflorum</i>	0	0	13
<i>Z.officinalae</i>	13	15	20
<i>P.amboinicus</i>	0	11	14
<i>P.nigrum</i>	0	12	15
Combination	11	13	16

Standard Antibiotic : Streptomycin ; Diameter of zone of inhibition- 29mm



extract increases. *Z.officinalae* showed maximum zone of inhibition and combination has moderate zone of inhibition against all the three bacteria. From this we can conclude that *Z.officinalae* has highest activity against *S. aureus*, *P. aeruginosa* and *K.pneumoniae*. Eventhough the herbal mixture is found to be little less active than *Z.officinalae* against the organisms tested, its activity compared to the individual plants *P. amboinicus*, *P.nigrum* and *O. tenuiflorum* is more. So the herbal mixture can be used to treat fever, cough, cold and other symptoms of respiratory tract infections because herbal mixture had shown activity against all the three bacterias at all the level of concentrations. From this study it is also found that *Z.officinalae* has more antimicrobial activity against the organisms tested as compared to other three plants that were used for this study. Since all the plant extracts were taken in equal ratio to prepare the mixture and *Z.officinalae* showed highest zone of inhibition on comparison, *Z.officinalae* is shown to be a potent antimicrobial agent.

Though *Z.officinalae* has higher antimicrobial activity, there is not enough literature support to prove its activity against fever, cold and other symptoms of respiratory tract infections. *Z.officinalae* is mainly used for GI related disorders. This herbal mixture is more efficient as it consists of *O.tenuiflorum* which is having antipyretic, analgesic and voice improving activity; *P.amboinicus* that provide relief from cough, asthma, bronchitis and other symptoms of respiratory tract infections. *P. nigrum* is used to improve appetite and digestion, as well as to treat intestinal gas, diarrhoea, cholera etc. *Z.officinalae* one of the component of the herbal mixture tested, effectively act against the causative organisms which is complimented by the antimicrobial activity of the other components. The other components of the mixture may be effective to treat the symptoms of respiratory infections as revealed by literature reviews.

Conclusion

The result of current study proved that all the four extracts and herbal mixture has antimicrobial activity. But the extract of *O.tenuiflorum* is not active against *Pseudomonas aeruginosa*.

When compared to gram negative bacteria, gram positive bacteria is more susceptible to the extracts. The herbal mixture showed activity against all the three test bacteria. But the herbal mixture showed less activity compared to the extract of *Z.officinalae*. The herbal mixture showed maximum zone of inhibition against *Staphylococcus aureus* and was found to be least active against *Klebsiella pneumoniae*. *Z.officinalae* one of the component of the herbal mixture tested, effectively act against the causative organisms which is complimented by the antimicrobial activity of the other components. The other components of the mixture may be effective to treat the symptoms of respiratory infections as revealed by literature reviews. These plants are easily available and possess wide range of biological properties and thus can be a source of low cost medication. Till now not much work has been done on the antiviral activity of this combination and thus there is a scope for scientific studies to fully exploit the medicinal properties of this combination to support traditional claims as well as, exploring some novel and promising drugs.

Acknowledgement

This project is a bridge between theoretical and practical working. First and foremost, we would want to thank "GOD ALMIGHTY". We are truly grateful to everyone who has supported us throughout the journey, especially our parents, teachers and all those who had contributed towards our success.

Conflict Of Interest

Nil

Funding

Nirmala College of Pharmacy, Muvattupuzha

Author Contribution

Dr Deepa Jose: Selected the topic and guided the project
Ms Sini Baby: Co-Guide Sujala Subash: Performed the experiment and prepared the article Gifty Lawrence, Aneesa Ayoob and Linta Jose: Performed the work and prepared the thesis

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