World Journal of Current Medical and Pharmaceutical Research
Content available at www.wjcmpr.com ISSN: 2582-0222

EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF ATHIMATHURAM CHOORANAM: AN IN VITRO STUDY

Rakshana.N[1], Umakanth.C[2], Chitra.U[3], Menaka. R[4], Sudhamathi Pushparaj. K[5]

[1]Department of PG Pothu Maruthuvam, Government Siddha Medical College, Chennai -106
[2]Department of Microbiology, Government Namakkal Medical College, Namakkal, 637 003, Chennai
[3,4,5]Department of PG Pothu Maruthuvam, Government Siddha Medical College, Chennai -106

Article History

Received on: 19-06-2025
Revised on: 09-07-2025
Accepted on: 24-08-2025

Abstract

Background: Infectious diseases remain a significant global health burden by rising threat to antibiotic resistance. According to Siddha literature, Athimathura Chooranam is a traditional herbal remedy that is used to relieve nausea, vomiting,head ache, Pitham and body itching. Aim/Objective The aim of the study is to investigate the Anti-microbial activity of Athimathura Chooranam against various micro-organisms and to determine its potential as a natural antimicrobial agent against various pathogens like Staphylococcus aureus (ATCC29213), Bacillus cereus(Clinical Cochin University), Klebsiella pneumonia(ATCC-700603),Escherichia coli(ATCC 25922)and Candida albicans (Clinical Cochin University). Methods Athimathura Chooranam was tested by agar-well diffusion method.The medium was prepared by using Mueller Hinton Agar and Sabouraud dextrose media which was subsequently autoclaved. Results The Anti-microbial activity of Athimathuram Chooranam was assessed against five microbial cultures: two Gram-positive bacteria (Staphylococcus aureus and Bacillus cereus), two gram-negative bacteria (Escherichia coli and Klebsiella pneumoniae), and one fungal Strain (Candida albicans). The Chooranam was tested at concentrations of 25mg/mL and 50mg/mL.It showed significant zone of inhibitions of 18 mm for Staphylococcus aureus,16mm for Bacillus cereus,24 mm for Klebsiella pneumoniae,25 mm for Escherichaicoli, and 23 mm for Candida albicans. This indicates that Athimathuram Chooranam exhibits antimicrobial properties against a broad Spectrum of microbial organisms.

Keywords: Athimathura Chooranam, Anti-microbial activity, Agar-well diffusion method, Zone of inhibition.

***Corresponding Author**
Rakshana.N

DOI: https://doi.org/10.37022/wjcmpr.v6i2.368

Introduction

Infectious diseases remain a significant global health burden by rising threat to antibiotic resistance [1]. According to Siddha literature, Athimathura Chooranam is a traditional herbal remedy that is used to relieve nausea, vomiting, head ache, Pitham and body itching. The Athimathura Chooranam is a herbal formulation with its reference Patharathagunavilakam [2].It contains Athimathuram (Glycyrrhiza glabra), Ginger (Zingiber officinale), Thipilli (Piper longum), Cardamom (Elettaria cardamomum), and Cumin seeds (Cuminum cyminum). The phytochemicals present in these plants hold strong promise for designing new herbal drugs and derivatives of these compounds are being generated to evaluate their pharmacological purposes. Natural products have been a prime source for the treatment of many ailments, many of which are consumed daily with diet. They provide significant protection against various diseases and disorders. Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bio-active constituents are triterpenoids, saponin, flavonoids, tannins, alkaloids, and phenolic compounds [3, 4]. Antimicrobial susceptibility testing can be used for drug discovery epidemiology and prediction of therapeutic outcome. Thus present study is aimed to screen the antibacterial and antifungal effect of siddha

herbal formulation Athimathura Chooranam against the selected human pathogens.

Aim and Objective

Aim

To evaluate the antimicrobial activity of Athimathura Chooranam against selected microbial pathogens through an in vitro study.

Objective

Primary Objective: To assess the antimicrobial efficacy of Athimathura Chooranam against clinical pathogens.

Secondary Objective: To determine the Zone of inhibition of the pathogens like SA: Staphylococcus aureus (ATCC-29213); BC Bacillus cereus (Clinical Cochin University); KP: Klebsiella pneumonia (ATCC-700603); EC: Escherichia coli (ATCC 25922) and CA: Candida albicans (Clinical Cochin University) at two different concentrations of 25mg/ml and 50mg/ml .

Materials and Methods

Materials

i) Athimathura Chooranam (polyherbal formulation)

ii) **Micro organisms** :Two gram positive bacteria: Staphylococcus aureus,Bacillus Cereus and two gram negative bacteria: Klebsiella Pneumonia, Escherichia coli,

iii) one fungal strain: Candida albicansiii) Nutrient Agar (NA medium)

iv) Sabouraud's Dextrose Agar

v) Distilled water

vi) Sterile petri dishes

vii) Incubatorviii) Autoclave

Methodology

Antibacterial Study

Chemicals and glassware

The solvent and chemicals used in this study were of analytical and laboratory grade and were procured from Merck chemicals. Media for this study were purchased from Hi-media. Glasswares made up of Borosil glass were used throughout the study. Growth media, glassware and other accessories were used after sterilization in an autoclave at 121 degree C at 15 lbs/sq. and inch pressure for 15 min.

Sterilization

The testing microbiology laboratory was sterilized by fumigation with formaldehyde and potassium permanganate for 24 hour every month. The inoculum hood was cleaned with 95% ethanol and exposed to UV light for 20 minutes before every use. The digital weighing balance was also cleaned with 95% ethanol and surgical gloves were used during the experiment for maintaining aseptic condition.

Collection of Test Microorganism

To evaluate the microbial studies, few cultures were procured from Clinical Laboratory in and around Chennai. The organisms used were Staphylococcus aureus (ATCC-29213), Bacillus cereus (Clinical Cochin University) (Gram positive), Escherichia coli (ATCC-25922), Klebsiella pneumonia (ATCC-700603) (Gram negative) and Candida albicans (Clinical Cochin University). All the organisms were confirmed using specific biochemical tests (Mackie and McCartney, 1996).

Medium Preparation

A lag phase uniform suspension of all the organisms listed above were prepared by inoculating a loopful of each culture in 10 ml of nutrient broth, incubated at 37ºC for about 6 to 8 hours.

Table: 01 Composition Mueller Hinton Agar (M-H Agar) Sigma-70191

Beef Infusion solids	**4.0**
Starch	1.5
Casein hydrolysate	17.5
Agar	15.0
Final pH 7.4±0.5	0.2 at 37ºC

Ingredients Grams/Litre 38 g of the media was suspended in 1000 ml of distilled water. Boiled to dissolve the medium completely and sterilized by autoclaving at 121°C for 15 minutes.

Table: 02 Sabouraud dextrose agar (SDA)

Dextrose (glucose)	40g
Peptone or mycological peptone	10g
Agar	15g
Final pH of 6.9±0.5	0.2at 35ºC

Suspend 65 g of the powder in 1000 ml of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. Sterilize in autoclave at 121°C for 15 minutes.

Sample preparation

10 g of Athimathurachooranam was dissolved in 50 mL of hydroalcohol (1:1) and the sample was kept in a shaking incubator for 24 to 48 hours. After complete solubility of the formulation the solution was filtered through whatman no.1 filter paper. The filtered extract was evaporated in a hot air oven at 40°C for one day. And the final extract were weighed and dissolved using the same solvent at 25 & 50 mg/mL concentration used for the further assay.

Antimicrobial activity of formulation

The Athimathurachooranam were tested for their antimicrobial activity using the Agar well diffusion method. All the cultures used for the study were obtained from Clinical Laboratory in and around Chennai. The Medium was prepared using Mueller Hinton Agar and Sabouraud dextrose agar (HiMedia), which was subsequently autoclaved. The autoclaved media was mixed thoroughly while still hot and then added to 100 mm Petri plates (25-30 ml/plate). Then, the bacterial strains that had been cultured for 24 hours were transferred to the medium. The wells, which were 10 mm in diameter and made from agar, were filled with 100 µl of diluted formulation at different concentrations (25 & 50 mg/mL) .The

commercially available drug Amp: Ampicillin 10mcg; Nx: Norfloxacin10mcg; Ap: Amphotericin 20mcg was used as control. The plates were then incubated at 37 °C for bacteria and 30 °C for fungus for 12 to 24 hours. After 24 hours of incubation, the test determines the efficacy of the formulation in terms of the zone of inhibition of the organism. The higher the zone of inhibition, the test sample will be more effective. Every experiment was performed twice (Balouiri et al 2016) [5].

Results

Antimicrobial activity

For antimicrobial study, a total of five microbial cultures were used in which two gram positive organisms namely S. aureus & B.cereus two gram negative organisms namely E. coli, K. pneumonia and one fungal strain C.albicans along. A significant growth of inhibition was shown against all the tested organisms indicating the profound potency of the Athimathurachoornam extract and the results were presented in Table 3& figure 1.

Table 3: Antimicrobial activity of Athimathurachoornam

Test samples	CONC (mg/ml)	*SA*	*BC*	*KP*	*EC*	*CA*
		(Zone of inhibition mm in dm)				
Athimadurachoornam	25	15	9	18	21	15
	50	18	16	24	25	18
	SC	-	-	-	-	-
	Std	-	15	21	-	12
Standard (Std)	Amp: Ampicillin 10mcg (Gram positive organisms); Nx: Norfloxacin10mcg (Gram negative organisms) ; Ap: Amphotericin 20mcg (Fungi)					
Organisms Abbreviation	*SA: Staphylococcus aureus* (ATCC-29213); *BC Bacillus cereus* (Clinical Cochin University); *KP: Klebsiella pneumonia* (ATCC-700603);*EC: Escherichia coli* (ATCC 25922)and *CA: Candida albicans* (Clinical Cochin University) SC-Solvent control					

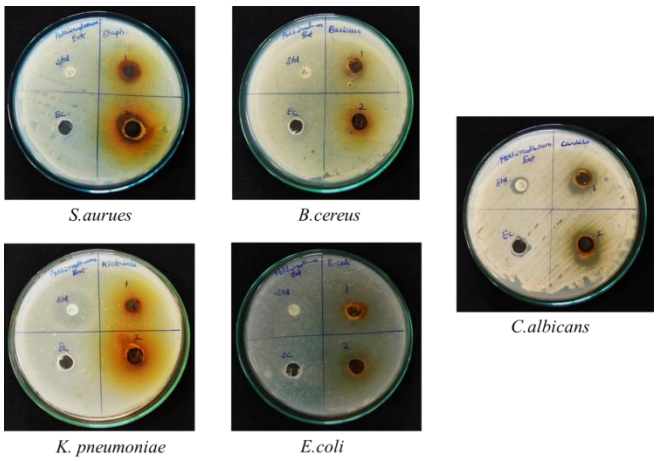

Figure 01:

Dicsussion

The antimicrobial susceptibility testing revealed that Escherichia coli was resistant to Norflaxacin 10mcg, (Escherichia coli with the zone of inhibition (25mm) at 50 mg/ml and 21mm at 25mg/ml) and also in Gram positive bacteria Staphylococcus aureus exhibited resitance to ampicillin whereas both organisms demonstrated sensitivity to Athimathura Chooranam (Staphylococcus aureus showed zone of inhibition of 18mm at 50 mg/ml and 15mm at 25mg/ml.) indicating its potential therapeutic application. The Staphylococcus aureus strain comes under methicillin resistant Staphylococcus aureus. Methicillin resistance has occurred in Staphylococcus aureus by mutation of penicillin-binding protein,a chromosome encoded protein.(Siddiqui A et al.,) [6]. Licorice scientifically named Glycyrrhiza glabra (Athimathuram) is widely used as a traditional medicine in india for multiple therapeutic activities such as cough, jaundice, polydipsia,peptic ulcer and burning micturition. Pharmacological studies exhibit the medicinal plant's anti-oxidant,anti-tumor, antibacterial and anti-HIV properties. Recent literature identified strong evidence of liquorice's therapeutic effects against COVID-19(koyuncu et al.,2021) A number of components isolated from Glycyrrhiza include glabridin, gabrin, glabrol, glabrene, hispaglabridin A, hispaglabridin B, 40-mrthylglabridin,and 3-hydroxyglabrol have exhibited potential in vitro antimicrobial activity [7]. The fresh ginger has the ability to balance the body's external conditions and promote sweating, which helps eliminate wind-cold-damp micro-organisms. Ginger's warming properties can specifically alleviate respiratory and pulmonary problems. Ginger can be used to treat illnesses characterized by vomiting caused by numerous circumstances and pathogenic agents [8]. Various bioactive-phytochemicals that are present in Thipilli including alkaloid, flavonoids , esters, and steroids were identified from the plant extracts and essential oils from root and fruits reported anti-inflammatory, analgesic, anthelminthic, antimicrobial, immunomodulatory, anti-asthmatic,cardio-protective and anti-snake venom agents [9]. Cardamom essential oil's anti-bacterial capabilities are effective against a wide spectrum of food-borne microbes. (Kubo et al.,1991). The antibacterial activity of CEO (10MG/mL) against Staphylococcus aureus was discovered. CEO possess potential broad-spectrum anti-bacterial and anti-fungal activity, which could be employed to reduce harm from food –borne pathogens and food spoilage species. The Disc-diffusion method has been used in majority of the studies.[10] Cuminum cyminum essential oils possessed antifungal activity against Botryis cinerea [11]. The present study findings highlights that Athimathura chooranam exhibits promising antimicrobial activity, suggesting its potential as a natural therapeutic agent against pathogenic organisms.

Conclusion

The findings suggest that Athimathura chooranam could serve as a potential natural alternative or complementary therapy to conventional antimicrobial agents. The study provides scientific evidence for the anti-microbial properties of Athimathura chooranam, bridging the gap between traditional knowledge and modern medicine. Further studies, including in

vivo evaluations, toxicological assessments to validate its clinical applications.

References

1. Kariuki S. Global burden of antimicrobial resistance and forecasts to 2050. Lancet. 2024;404(10459):1172–3.
2. Pillai KN. Pathartha Guna Vilakkam. Chennai: Rathina Nayakar and Sons; [Year not provided].
3. Hill AF. Economic botany: a textbook of useful plants and plant products. 2nd ed. New York: McGraw Hill; 1952.
4. Collee JG, Fraser AG, Marmion BP, Simmons A. Mackie and McCartney Practical Medical Microbiology. 14th ed. New York: Churchill Livingstone; 1996.
5. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 2016;6(2):71–9.
6. Siddiqui A, Koirala J. Methicillin resistant Staphylococcus aureus (MRSA) [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 [cited 2025 Sep 5]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482221/
7. Sekaran K, Karthik A, Varghese RP, Sathiyarajeswaran P, Shree Devi MS, Siva R, et al. In silico network pharmacology study on Glycyrrhiza glabra: Analyzing the immune-boosting phytochemical properties of Siddha medicinal plant against COVID-19. In: Medicinal Plants for COVID-19 and Related Viral Infections. Academic Press; 2023. p. 233–55.
8. Zhang C, Rao A, Chen C, Li Y, Tan X, Long J, et al. Pharmacological activity and clinical application analysis of traditional Chinese medicine ginger from the perspective of one source and multiple substances. Chin Med. 2024;19(1).
9. Biswas P, Ghorai M, Mishra T, Gopalakrishnan AV, Roy D, Mane AB, et al. Piper longum L.: A comprehensive review on traditional uses, phytochemistry, pharmacology, and health-promoting activities. Phytother Res. 2022 Oct 18.
10. Lawrence V, Pandian A, Somasundaram A, Pandian R, Saratha V, Ashokkumar K, et al. Phytochemical analysis and pharmacological potential of cardamom. [Journal name not provided]. 2021.
11. Al-Snafi AE. The pharmacological activities of Cuminum cyminum – A review. IOSR J Pharm. 2016;6:46–65.