INTERNATIONAL JOURNAL OF DRUG DISCOVERY AND HERBAL RESEARCH (IJDDHR)
1(4): October -December: (2011), 224-230

ISSN: 2231-6078

Research Article

Antibacterial Activity of *Thaaleesaadhi Chooranam* Against Human Pathogens

T.G.Nithya*,[1] V.Dahineeswari,[1] Shirlie Roy Chowdhury,[1] S.Sivakumar [2]

1. Department of Biotechnology, Faculty of Science and Humanities, SRM University, Kattankulathur.
2. Department of Gunapadam , National institute of Siddha, Tambaram Sanitorium, Chennai.

Abstract

Thaaleesaadhi chooranam is a polyherbal formulation prepared from 23 different herbs being used for treatment of various *kapha* (*in tamil*) diseases and found to have antibacterial,antitumour,antioxidant,anti-inflammatory, anti- cancer, anti-viral and analgesic properties.In the present study the antibacterial activity of *thaaleesaadhi* chooranam were screened.The aqueous and ethanolic extracts of *thaaleesaadhi* chooranam were tested for antibacterial activity against selected human pathogens viz. *S.aureus, P.aeruginosa, B.subtilis ,K.pneumoniae and E.coli*. The results of antibacterial activity revealed that the extracts showed good inhibitory activity against all test pathogens.

Key Words: Polyherb, siddha, *thaaleesaadhi* antibacterial, disc diffusion.

Introduction

In the present era, plant and herb resources are abundant, but these resources are dwindling fast due to the onward march of civilization [1]. Although a significant number of studies have been used to obtain purified plant chemical, very few screening programmes have been initiated on crude plant materials. It has also been widely observed and accepted that the medicinal value of plants lies in the bioactive phytocomponents present in the plants[2].

According to World Health Organization, medicinal plants are the best source to obtain a variety of newer herbal 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 understand their properties, safety and efficacy[3].In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many kinds of diseases have been treated with herbal medications throughout the history of mankind.Siddha is the oldest healing system of medicine and it has fundamental aspects for drug formulation.Major formulations used in Siddha are based on herbs.The medicinal herbs are used as decoctions, infusions, tinctures, and powders[4].

***Corresponding Author**
E-mail: nithya_245@yahoo.co.in
Mob. +919841404193

When two or more herbs are used in formulations, they are known as polyherb. The therapeutic value of medicinal plants depends upon the presence of one or more constituents possessing certain physiological and pharmacological activity. The main herbs are selected according to the disease; other herbs are used to enhance the effects of chief herb [5] .

There is currently a large and ever expanding global population base that prefers the use of natural products in treating and preventing medical problems because herbal plants have proved to have a rich resource of medicinal properties [6].The different herbs encountered at different herbal homes have different medicinal properties and many of them have multiple uses and hence used for the management of more than one ailment [4]. Many commercially proven drugs used in modern medicine were initially used in crude form in traditional or folk healing practices or for other purposes that suggested potentially useful biological activity[7].

Microorganisms are the causative agents of almost all kinds of acute and chronic diseases. Plants based antibacterials have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antibacterials. The use of plant extracts with known antibacterial properties, can be of great significance in therapeutic treatments.Although hundreds of plant species have been tested for antibacterial properties, the vast majority of them have not been adequately evaluated [8].Considering the vast potentiality of plants as sources for antibacterial drugs with reference to antibacterial agent, a systematic investigation was undertaken to screen the polyherbal formulation *Thaaleesadhi* chooranam for antibacterial activity against selected human pathogens.

Thaaleesaadhi chooranam is a polyherb with 23 different herbs composition like (in Tamil) *Thaaleesa pathiri, Lavanga pattai, Nellikai, seeragam, Milaku, Omam, Thippilli, Perungayam etc.*,.(Table 1).The powder form of this sidhha Chooranam is used to treat cough,cold,phlegamatic conditions,pneumonia and a wide range of *kapha disease* and ear disease.Analysing the phytochemicals and evaluating the anti microbial properties in medicinal plants provides scientists with insight to know how they are medicinally effective whereas understanding the chemical composition leads to the development of new medicines. The use of alchocol or water as solvent is efficient in extracting a wide variety of active components Therefore, we analysed the

Thaaleesadhi chooranam for the presence of phytochemicals and evaluated the antibacterial activity against selected human pathogens(Table 2).

Material and Methods

Preparation of Formulation:

The ingredients were procured from commercial Siddha raw drug store and was authenticated and prepared by Dr.Sivakumar. (Table 1). All the ingredients were shade dried, powdered and mixed thoroughly in same proportion.The mixture was further boiled in distilled water at 100ºC for 60 minutes and filtered.The filtrate was evaporated to dryness, used for subsequent experiments and they are designated as Chooranam since they are comprised of multiple herbs.

Solvent extract preparation:

10 g of air dried *Thaaleesaadhi* chooranam powder was extracted with 100ml of Organic solvent (Ethanol) and kept on rotary shaker at 190-220 rpm for 24 hours.The supernatant was collected and solvent was evaporated to make the final volume one – fourth of the original volume and stored at 4^0C in air tight bottles [9].

Aqueous extract preparation:

The aqueous extract is prepared by soaking 100grams of *Thaleesaadhi* Chooranam powder in 200 ml of distilled water for 12hours.The extracts were filtered using Whatman filter paper (125 mm) [10].

Phytochemical screening:

The qualitative tests were carried out in both the extract of *Thaleesaadhi* chooranam using standard procedures [11],[12], [9].

Growth and Maintenance of Test Microorganism for Antibacterial Studies:

Bacterial cultures of *Bacillus subtilis (B. subtilis), Escherichia coli (E. coli), Pseudomonas aeruginosa (P.aeruginosa),Staphylococcus aureus (S. aureus) and Klebsiella pneumoniae (K.pneumoniae)*were obtained from Department of Microbiology, SRM Medical College, India, and used for antibacterial studies. The bacteria were maintained on Nutrient Agar(NA) slants at 4°C.For further study ,cultures have been grown in Nutrient Broth (NB) for 24hrs as overnight cultures.

Disc diffusion Method:

The antibacterial assay of aqueous and ethanolic extracts was performed by Disc diffusion method[13].The Nutrient agar media (20ml) was poured into sterilized petri dishes and left to solidify at room temperature. The overnight bacterial cultures have been spread plated on these petridishes using sterile L rod.Taking the crude extract concentration as 100%, different concentration of aqueous and ethanolic extracts was prepared viz 20%,40%, 60%, and 80%. Whattman's No.1 filter paper discs (3mm) were soaked in 0.1 ml of Ethanol extract of varying concentrations from 20% -80%.The similar procedure was carried out for aqueous extract with varying concentration from 20% -80% simultaneously. The filter paper discs were placed equidistantly on inoculated media and diffusion of solution was allowed to occur for 30 minutes at room temperature. Plates were incubated at 37˚C for 24 hours. The average zone of inhibition was recorded. Sterile distilled water and Ethanol were maintained as control. The diameters of the inhibition zones were measured in mm.

Results

The qualitative analysis of the Chooranam extract showed the presence of phytochemical constituents such as Alkaloids, Cardiac glycosides, Flavanoids, Steroids and Tannins except Saponin (Table 3).Antibacterial activity results obtained in the present study revealed that the tested extracts posses potential antibacterial activity against *B. subtilis, S. aureus ,E. coli,K.pneumoniae ,P.auregenosa*(Table 4).The diameters of the inhibition zones against all the tested bacteria were measured in mm. When tested by the disc diffusion method, the ethanol extracts of *Thaaleesadhi chooranam* showed significant activity against *E.coli. S.aureus, K.pneumoniae around 12mm.*The highest antibacterial activity of 14 mm was observed in *B. subtilis* and least activity was recorded in *P.auergenosa* of 8 mm.The range of zone of inhibition by ethanolic extracts against pathogens were in the following range of higher to lower *viz., B. subtilis, S. aureus ,E. coli, K.pneumoniae ,P.auregenosa*(Table 5 & Fig.,1).The antibacterial results for aqueous extract of *Thaaleesadhi chooranam* showed maximum activity against *B. subtilis* of 12 mm and least activity observed in *P.aueregenosa of 6 mm* .Inhibitory activity against *E.coli. S.aureus, K.pneumoniae was around 9-10mm* (Table 6 & Fig.,2).The results elucidated that the activity was higher against *Gram +ve strains* than *Gram –ve* pathogens.On comparison of both the extracts the activity against pathogens showed similar results with effective and significant inhibitory action(Table 7 & Fig.,3).

Discussion

Antibiotics provide the main basis for the therapy of bacterial infections. However, the high genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance. Thus there has been a continuing search for new and more potent antibiotics[14]. According to World Health Report on infectious diseases 2000, overcoming antibiotic resistance is the major issue of the WHO for the next millennium.Hence,the last decade witnessed an increase in the investigations on plants as a source of human disease management[15]and not many reports are available on the exploitation of plants for the management of plant diseases[16]. This is mainly due to lack of information on the screening and evaluation of diverse plants for their antibacterial potential.

Table 1: Composition of Thaaleesadhi Chooranam (in 100 gms)

S.NO	Siddha Name	Botanical Name	Family Name	Quantity
1	Thaaleesa pathiri	*Taxus beccata*	*Taxaceae*	4.54gms
2	Lavanga pattai	*Cinnamomoum zeylanicum*	*Myrtaceae*	4.54gms
3	Elakkai	*Eletteria cardamomum*	*Zingiberaceae*	4.54gms
4	Chukku	*Zingifer officinale*	*Zingiberaceae*	4.54gms
5	Adhimadhuram	*Glycyrrhiza glabra*	*Fabaceae*	4.54gms
6	Perungayam	*Ferula asafetida*	*Apiaceae*	4.54gms
7	Nellikai	*Emblica officinalis*	*Phyllanthaceae*	4.54gms
8	Kostham	*Saussurea lappa*	*Asteraceae*	4.54gms
9	Thippili	*Piper longum*	*Piperaceae*	4.54gms
10	Seeragam	*Cuminum cyminum*	*Apiaceae*	4.54gms
11	Sadakuppai	*Anethum sowa*	*Apiaceae*	4.54gms
12	Karunseegram	*Nigella sativa*	*Ranunculaceae*	4.54gms
13	Thippili moolam	*Piper longum*	*Piperaceae*	4.54gms
14	Lawangam	*Syzygium aromaticium*	*Myrtaceae*	4.54gms
15	Millaku	*Piper nigrum*	*Piperaceae*	4.54gms
16	Jaadhikai	*Myristica fragrans*	*Myristicaceae*	4.54gms
17	Sadaamanjil	*Nardostachys*	*Valerianaceae*	4.54gms
18	Sirunaga poo	*Cinnamomom verum*	*Lauraceae*	4.54gms
19	Shenbaga mokku	*Michelia champaca*	*Magnoliaceae*	4.54gms
20	Vaividangam	*Embelia ribes*	*Myrsinaceae*	4.54gms
21	Omam	*Trachyspermum ammi*	*Apiaceae*	4.54gms
22	Kothamalli	*Coriandrum sativum*	*Apiaceae*	4.54gms
23	Sugar	*Saccharum officinuram*	*Poaceae*	0.1 gms

Table 3 :Phytochemical screening results of Polyherb -*Thaaleesaadhi Chooranam*

Compound	Result
Alkaloids	+
Cardiac glycosides	+
Flavonoids	+
Steroids	+
Saponins	--
Tannins	+

+ = Positive -- = Negative

Therefore, in the present investigation of *Thaleesaadhi* Chooranam,a multiherbal formulation was evaluated for its antibacterial potential for the first time against selected human pathogenic bacteria which are known to cause many infectious diseases.

Exogenous chemical and endogenous metabolic processes in the human body or in the food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage. Drugs with multiple mechanisms of protective action, including antioxidant properties, antibacterial may be one way forward in minimizing tissue injury in human disease[17]. This polyherb composition is effective in treatment of various diseases as they are rich in essential phytochemicals.Moreover, the herbs present in *Thaleesaadhi* Chooranam are readily available products and were regularly used in Indian food preparation, such as *Rasam* (Tamil), *Kasayam* (Tamil), and are of major components in South Indian Cooking.

The combination of multiple herbs has increased the efficacy of the *Thaleesaadhi* Chooranam to a greater extent, since they possess medicinal values exerted by the presence of phytochemicals.Flavonoids are known to be synthesized by plants in response to microbial infection. Hence it should not be surprising that they have been found to be effective as antibacterial substances against a wide array of infectious agents[18].Tannins are also known as antibacterial agents.They are water-soluble polyphenols and precipitated proteins present in many plant foods.Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein.The growth of many fungi, yeasts, bacteria, and viruses were inhibited by this compound[19].Alkaloids can be used for treating Cough and has anti-tumour, analgesic property and Steroids contains anti-inflammatory activity(Table 8).

Conclusion

Medicinal plant products still remain as the primary source of supply of many important drugs in orthodox medicine today. Since there are so many of these naturally occurring substances of plant origin, it is obvious that the plant kingdom offers a better opportunity of providing useful medicinal compounds for the treatment of numerous challenging diseases. Elucidating the chemical structure of active components of herbs also makes room for synthetic modifications for better pharmacokinetic profiles. In this study a polyherbal formulation was evaluated for its phytochemical and antibacterial activity.Presence of significant secondary metabolites presence were recorded. Aqueous and methanol extracts of the formulation were found to be more effective against test pathogens.

Acknowledgements

I sincerely acknowlede SRM Medical college for providing us with Bacterial cultures and for their co-operation .I also indeptly acknowledge our Director Dr.R.Balasubramanium,FSH,SRM University for his encouragement and support.

References

1) Vogel HG.1991, Similarities between various systems of traditional medicine. Considerations for the future of ethnopharmacology. *J Ethnopharmacol* 35:179–90.

2) Veermuthu, D., Muniappan, A. and Savarimuthu, I. 2006. Antibacterial activity of some ethnomedicinal plants used by Paliyar tribe from Tamilnadu, India. *BMC Complementary and Alternate Medicine* 6(35).

3) J.K. Mensah, R I. Okoli ,1 A. A. Turay,2: Phytochemical Analysis of Medicinal Plants Used for the Management of Hypertension: *Ethnobotanical Leaflets* 13: 1273-87. 2009.

Table 2: Pathogenicity of selected Human pathogens

Organisms Tested	Gram Staining	Pathogenicity
E.coli	*Gram negative*	Gastroenteritis,Urinarytrack infections
S.aureus	*Gram positive*	Skin and soft tissue infections such as abscesses, furuncles, and cellulitis
P.aeruginosa	*Gram negative*	Respiratory system infections, dermatitis, soft tissue infections, bone and joint infections, gastrointestinal infections
B.subtilis	*Gram positive*	Food poisoning
K.pneumoniae	*Gram negative*	Pneumonia

4) Kartik Ch.patra :standardisation of siddha formulation. *Indian Journal of Traditional Knowledge* Vol.8;July 2009

5) Gandhiraja, N S. Sriram,* V. Meenaa, J. Kavitha Srilakshmi, C. Sasikumar and R.Rajeswari Phytochemical Screening and Antibacterial Activity of the Plant Extracts of Mimosa pudica L. Against Selected Microbes, *Ethnobotanical Leaflets* 13:618-24, 2009.

6) Retnam K , De Britto A; Antibacterial activity of a medicinal plant Hybanthus enneaspermus (Linn) F.Muell. *N. Prod. Rad*. 2007; 6(5):366-368

7) Balandrin, M.F., J.A. Klocke, E.S. Wurtele and 2006. Phytochemical analysis and antibacterial W.H. Bollinger, 1985. Natural plant chemicals: activity of *Oxalis corniculata*: A known medicinal Sources of Industrial and Medicinal materials. plant. My. Science., 1: 72-78. Science, 228: 1154-1160

8) Harborne J B;*Phytochemical Methods*: *A Guide To Modern Technique of Plant Analysis*.3rd ed .London, England: Chapman and Hall; 1998:114-118.

9) H.O. Edeoga, D. E. Okwu, Phytochemical constituents of some Nigerian medicinal Plants; African Journal of Biotechnology Vol. 4 (7), pp. 685-688 July 2005

10) Sofowora AE (1993). Medicinal Plants and Traditional Medicines inAfrica.. 2nd edition. Spectrum Books, Ibadan, Nigeria. p. 289.

11) Trease E.G., Evans W.C 1978. Pharmacognosy. 11th Edition, Balliere Tindall, London. 115-222.

12) Bauer, A.W., Kirby, W.M.M., Sherris, J.C., Turck, M., 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45: 493-496.

13) Heisig P. 2001. *Planta Medica*. 67: 4–12.

14) Aiyelaagbe *et al*. 2000. *Fitoterapia.* 72: 544–546.

15) Satish S., Raveesha K. A. and Janardhana G. R. 1999. *Lett. Applied Microbiol.* 28: 145–147.

16) Barry H. Antioxidant effects, a basis for drug selection, *Drugs* 1991; 42: 569-573.

17) Jasmine. R, Daisy.P and Selvekumar. B.N., 2007. *In vitro* Efficacy of Flavonoids from *Eugenia jambolana* Seeds Against ESL-Producing Multidrug-Resistant Enteric Bacteria. Research Journal of Microbiology. 2 (4):369–374.

18) Prasad, N. R.,et al., 2008. Preliminary phytochemical screening and antibacterial activity of *Samanea saman* . Journal of Medicinal Plants Research. 2: 268-270.

Table 4 :Antibacterial screening of the extracts showing the range of zone of inhibition (mm)

Test Organism	Ethanol Extract	Aqueous Extract
E.coli	4 to 12	3 to 10
S.aureus	8 to 12	6 to 10
P.aeruginosa	4 to 8	4 to 6
B.subtilis	6 to 14	5 to 12
K.pneumoniae	4 to 12	4 to 9

Table 5 :Zone of inhibition of Ethanolic extract (mm)

Test Organism	Zone of Inhibition for Ethanol Extract in %			
	20%	40%	60%	80%
E.coli	4±0.14	7±0.11	9±0.10	12±0.14
S.aureus	8±0.12	9±0.12	10±0.15	12±0.13
P.aeruginosa	4±0.00	4±0.10	6±0.10	8±0.13
B.subtilis	6±0.11	8±0.14	12±0.17	14±0.16
K.pneumoniae	4±0.14	5±0.12	8±0.13	12±0.12

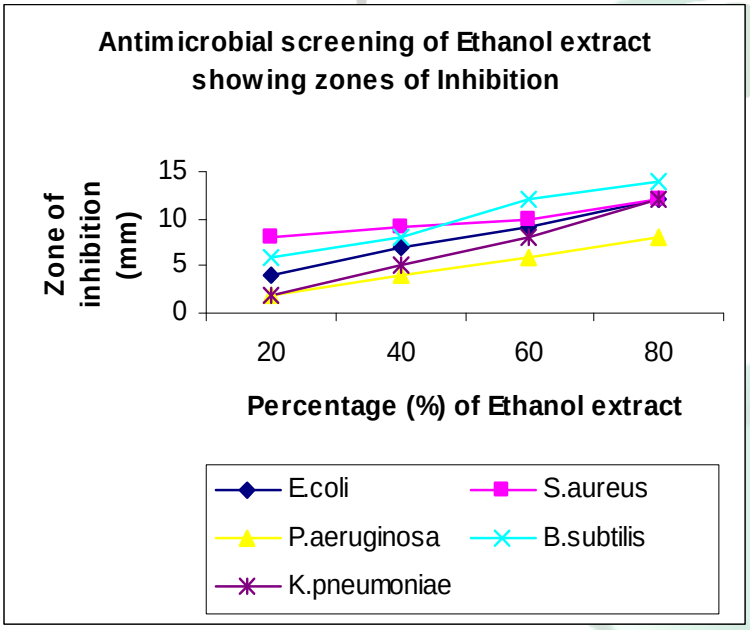

Figure:1

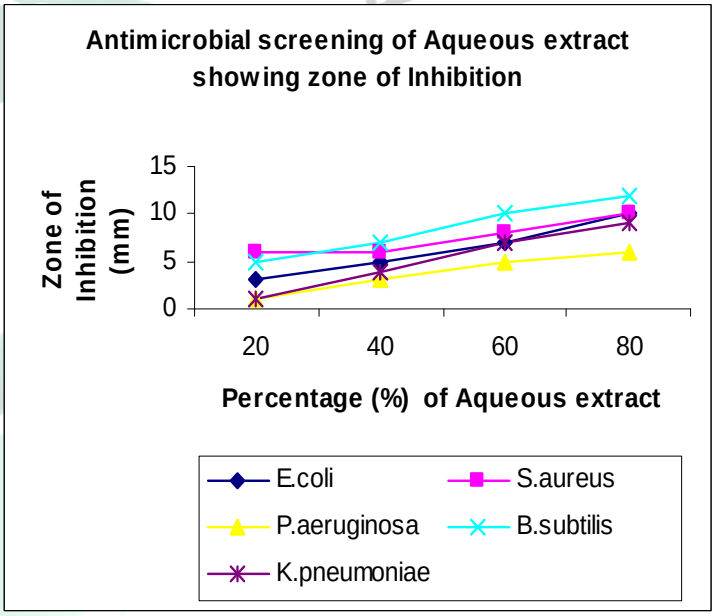

Figure:2

Table 6 : Zone of inhibition of Aqueous extract (mm)

Test Organism	Zone of Inhibition for Aqueous Extract in %			
	20%	40%	60%	80%
E.coli	3 ± 0.13	5±0.14	7±0.12	10±0.15
S.aureus	6 ± 0.12	6±0.15	8±0.12	10±0.15
P.aeruginosa	4 ± 0.11	3±0.12	5±0.13	6±0.14
B.subtilis	5±0.13	7±0.12	10±0.12	12±0.17
K.pneumoniae	4±0.12	4±0.12	7±0.12	9±0.13

Table 7: Comparison of Antibacterial activity of Ethanol and Aqueous extracts.

Concentration of Extracts in % with Zones of Inhibition(in mm)

Organisms	20%E	20%A	40%E	40%A	60%E	60%A	80%E	80%A
E.coli	4±0.14	3±0.13	7±0.11	5±0.14	9±0.10	7±0.12	12±0.14	10±0.15
S.aureus	8±0.12	6±0.12	9±0.12	6±0.15	10±0.15	8±0.12	12±0.13	10±0.15
P.aeruginosa	4±0.00	4±0.11	4±0.10	3±0.12	6±0.10	5±0.13	8±0.13	6±0.14
B.subtilis	6±0.11	5±0.13	8±0.14	7±0.12	12±0.17	10±0.12	14±0.16	12±0.17
K.pneumoniae	4±0.14	4±0.12	5±0.12	4±0.12	8±0.13	7±0.12	12±0.12	9±0.13

E = Ethanol extracts A = Aqueous extracts

Table 8: Medicinal uses of Phytochemicals

Phytochemicals	Medicinal uses
Alkaloids	Treatment of Cough, Anti-tumour and analgesic activity
Flavanoids	Anti-oxidant and anti- cancer activity
Cardiac glycosides	Treatment of cardiac diseases
Tannins	Anti-viral,Anti-bacterial activity
Steroids	Anti -inflammatory activity

Figure:3