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Antibacterial activity of *Vallarai* Chooranam against Human Pathogens

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Abstract: *Vallarai* chooranam is a polyherb,comprised of 10 different herbs,used for treatment of diabetics,urinary tract infection,leucorrhea,veneral disesase and also to improve memory power.The present investigation was carried out to study the unexplored area of the *Vallarai* chooranam towards their antibacterial activity against both Gram positive and Gram negative organisms by Disc diffusion method.Aqueous and solvent extracts of the chooranam were tested against selected human pathogens *viz. S.aureus, P.aeruginosa, B.subtilis ,K.pneumoniae and E.coli*. Both the extracts were found to be more effective against all the test pathogens.*B.Subtilis* was more susceptible to the aqueous extracts among the tested organisms.The results of antibacterial activity revealed that both the extracts exhibited good inhibitory activity against all test pathogens.The presence of phytochemicals in the formulation were also assayed.The results showed positive response for significant secondary metabolites.
Key words: Polyherbal, antibacterial,Qualitative analysis,Aqueous extract,Solvent extract,*Vallarai* Choornam.

Introduction:

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and fewer side effects. Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease[1]. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions [2].

It has been reported that there has been an alarming increase in number of diseases and disorders caused by synthetic drugs prompting a switch over to traditional herbal medicine[3].The earliest record of human civilization and culture of China, Egypt, Assyria, and Indies valley reveals that the elders and wise men of those times used herbs to treat various diseases. Many of the indigenous medicinal plants are used as spices for cooking[4].The ancient use of plants for healing purposes forms the origin of much of modern medicine. Many traditional drugs originate from plant sources: a century ago, most of the effective drugs were plant based [5].

It is estimated that there are 250,000 to 500,000 species of higher plants on earth. But relatively small

percentage (5-15%) has been systematically investigated for the presence of bioactive compounds[6]. Microorganisms are the causative agents of almost all kinds of acute and chronic diseases.

Plants based antimicrobials 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 antimicrobials.The use of plant extracts with known antimicrobial properties, can be of great significance in therapeutic treatments.Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of them have not been adequately evaluated[7].Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals,and plants.One of such resources is folk medicines. Systematic screening of them results in the discovery of novel effective compounds [8].The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies[9].Considering the vast potentiality of plants as sources for antimicrobial drugs with reference to antibacterial agent, a systematic investigation was undertaken to screen the polyherbal formulation *Vallarai* chooranam for its antibacterial activity against selected human pathogens.

This polyherbal formulation is a composition of 10 different herbs viz.,(*In tamil*) *Vallarai, Jaadhikai, Jaadhipathiri,Lawangam,Elakkai,Thaaleesapathiri,Maasikkai,Kadukkai,Nellikai,Thaantrikkai*.**(Table 1).**The powder form of this sidhha Chooranam is used to treat diabetics,Urinary tract infection,Leucorrhea,Veneral disesase and also used to improve memory power and blood purification[10].The use of alchocol or water as solvent is efficient in extracting a wide variety of active components. Therefore, we analysed the *Vallarai* chooranam for the presence of phytochemicals and evaluated the antimicrobial activity against selected human pathogens.

Materials and Methods:

Preparation of Formulation:

The ingredients were procured from reputed commercial Siddha supplier (Dr.Sivakumar and Dr.Juliet, Selaiyur.Chennai) and authenticated [**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 grams of air dried *Vallarai* 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[11].

Aqueous extract preparation:

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

Table 1:Polyherbal formulation of *Vallarai* chooranam

S.No	Siddha Name	Botanical Name	Quantity	Family Name
1	Vallarai	*Centella asiatica*	50gms	*Mackinlayaceae*
2	Jaadhikai	*Myristica fragrans*	10gms	*Myristicaceae*
3	Jaadhipathiri	*Myristica fragrans*	10gms	*Myristicaceae*
4	Lawangam	*Syzgium aromaticum*	10gms	*Myrtaceae*
5	Elakkai	*Eletteria cardamomum*	10gms	*Zingiberaceae*
6	Thaaleesa pathiri	*Taxus beccata*	10gms	*Taxaceae*
7	Maasikkai	*Quercus infectoris*	10gms	*Fagaceae*
8	Nellikai	*Emblica officinalis*	10gms	*Phyllanthaceae*
9	Kadukkai	*Terminalia chebula*	10gms	*Combretaceae*
10	Thaantrikkai	*Terminalia belerica*	10gms	*Combretaceae*

Phytochemical screening :

The qualitative tests were carried out in both the extract of *Vallarai* chooranam using standard procedures[13-15].Both the extacts were analysed for the presence of significant secondary metabolites *viz* Alkaloids, Tannins, Flavonoids ,Cardiac glycosides, Steroids and Saponins.

Growth and Maintenance of Test Microorganism for Antimicrobial 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 the studies were performed at Department of Biotechnology,FSH,SRM University.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[16].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.

Table 2 :Antibacterial screening of the extract showing the range of zone of inhibition (mm)

Test Organism	Ethanol Extract	Aqueous Extract
E.coli	3 to 11	2 to 11
S.aureus	6 to 12	3 to 10
P.aeruginosa	2 to 7	2 to 6
B.subtilis	7 to 14	7 to 14
K.pneumoniae	4 to 12	4 to 9

Table 3 :Zone of inhibition of Ethanolic extract (mm)

Test Organism	Zone of Inhibition (mm) by varying concentration(%)			
	20%	40%	60%	80%
E.coli	3.12±0.12	6.22±0.15	8.27±0.13	11.22±0.12
S.aureus	6.53±0.11	10.16±0.10	11.13±0.16	12.15±0.19
P.aeruginosa	2.32±0.10	3.85±0.17	5.61±0.13	7.16±0.18
B.subtilis	7.21±0.13	9.45±0.20	11.33±0.19	14.33±0.16
K.pneumoniae	4.33±0.20	6.27±0.14	9.13±0.10	12.33±0.12

Graph 1:

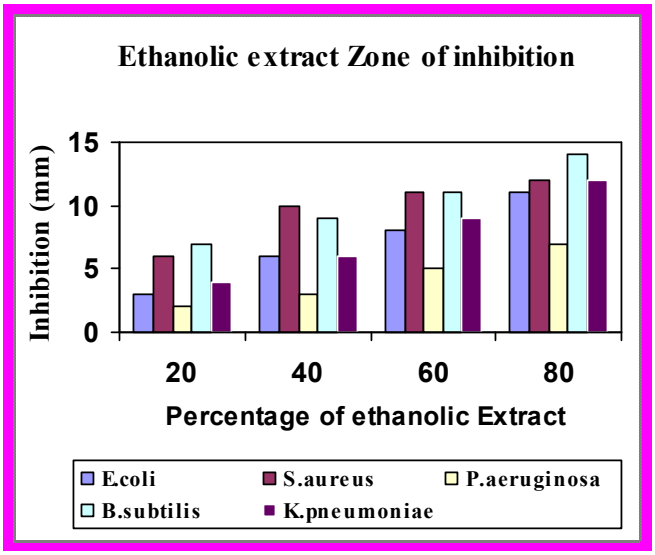
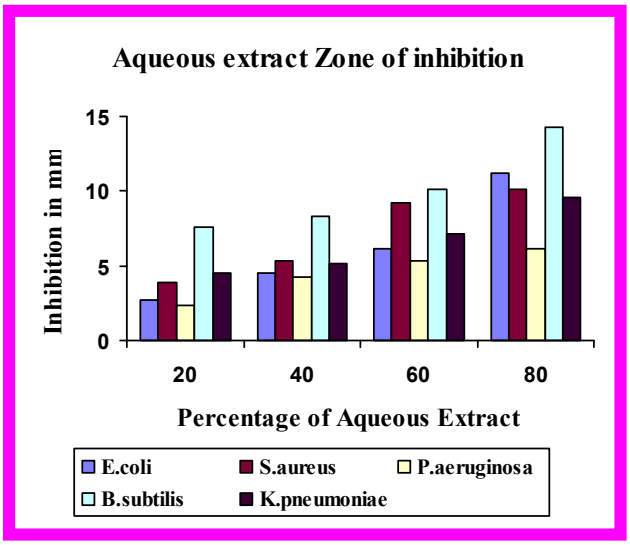


Table 4 : Zone of inhibition by Aqueous extract (mm)

Test Organism	Zone of Inhibition (mm) by varying concentration(%)			
	20%	40%	60%	80%
E.coli	2.75± 0.11	4.51±0.14	6.13±0.13	11.24±0.16
S.aureus	3.87 ± 0.14	5.31±0.15	9.19±0.11	10.16±0.17
P.aeruginosa	2.37 ± 0.20	4.22±0.12	5.31±0.12	6.15±0.18
B.subtilis	7.62±0.15	8.32±0.12	10.14±0.14	14.31±0.20
K.pneumoniae	4.53±0.14	5.12±0.15	7.16±0.13	9.56±0.15

GRAPH 2:



GRAPH 3:

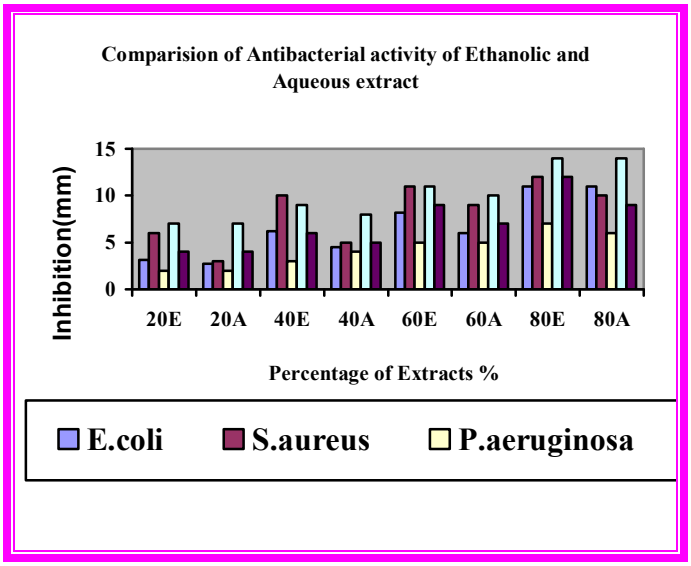


Table 5: Phytochemical screening results of *Vallarai* Chooranam

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

+ = Positive

Results :

Antimicrobial 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 2).The diameters of the inhibition zones against all the tested bacteria were measured in mm. The results showed that increase in concentration of extract increased the zone of inhibition .When tested by the disc diffusion method, the Ethanolic extracts of *Vallarai Chooranam* showed significant activity against *S.aureus, K.pneumoniae around 12mm*.The zone of inhibition for *E.coli* was observed as 11 mm.The highest antibacterial activity of 14 mm was observed in *B. subtilis* and least activity was recorded in *P.auergenosa* of 7mm.The range of zone of inhibition by ethanolic extracts against pathogens were in the following order of higher to lower,*viz.,B.subtilis,S.aureus, E.coli, K.pneumoniae, P.auregenosa*(Table:3&Graph1).

The antimicrobial results for aqueous extract of *Vallarai Chooranam* showed maximum activity against *B. subtilis* of 14 mm and least activity observed in *P.aueregenosa of 6 mm* .Inhibitory activity against *E.coli. S.aureus, K.pneumoniae was around 9-11mm* (Table 4 & Graph 2).The results elucidated that the activity was higher against *Gram +ve strains* than *Gram –ve* pathogens.In classifying the antibacterial activity as Gram-Positive or Gram-negative, it would generally be expected that a much greater number would be active against Gram-positive than Gram-negative bacteria[17], however, in our study both Gram positive and Gram negative bacteria were inhibited by the extracts of the *Vallarai Chooranam.* On comparison of both the extracts the activity against pathogens showed similar results with effective and significant inhibitory action(Graph 3).The aqueous and solvent extracts were used to identify the presence of various phytochemicals .Standard test for Flavonoids, Alkaloids, Saponins, Cardiac glycosides, Tannins and Steroids showed positive response(Table 5).

Discussion:

Ayurveda is a traditional Indian Medicinal System practiced for thousands of years. Considerable research on pharmacognosy, chemistry, pharmacology and clinical therapeutics has been carried out on ayurvedic medicinal plants. The polyherbal formulations described in Ayurveda have been the basis of treatment of various human diseases. Biological evaluation of herbal formulations based on their medicinal uses forms the basis for development of new drugs from plants[18]. Historically, plants have provided a source of inspiration for novel drug compounds,as plant derived medicine have made large contribution to human health and well-being.

The combination of multiple herbs has increased the efficacy of the *Vallarai* 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[19].Tannins (commonly referred to as tannic acid) are also known as antimicrobial 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 Tannins.[20]

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[21]and not many reports are available on the exploitation of plants for the management of plant diseases[22].This is mainly due to lack of information on the screening and evaluation of diverse plants for their antibacterial potential.Therefore, in the present investigation of *Vallarai* 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.

Conclusion:

In the present investigation *Vallarai* Chooranam was evaluated for its antibacterial potential for the first time against selected human pathogenic bacteria which are known to cause many infectious diseases and also the phytoanalysis revealed the presence of medicinally active constituents. The antibacterial activity of this polyherbal formulation would help for development of a new alternative medicine system which has no side effects. Both the extracts of this formulation possess a broad spectrum of activity and open the possibility of finding new clinically effective antimicrobial compounds.Hence the study has provided biochemical basis for ethanopharmacological claims of the Chooaranam in treatment and prevention of various diseases and disorders.

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