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Research Article

ANTIBACTERIAL AND ANTIOXIDATION ASSAY OF *THALIPATHIRI* CHOORANAM PRESCRIBED TO CURE COUGH BY THE CHERUTHIKONAM TRADITIONAL SIDDHA MEDICINAL PRACTITIONER OF KANYAKUMARI DISTRICT, INDIA

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ABSTRACT

Objective: The main objective of the present investigation is to evaluate the phytochemical constituents, antioxidant potential and antibacterial activity of Siddha Cough Chooranam prescribed by the Traditional Practitioner to ensure the effect of medicine.

Methods: Phytochemical analysis of the Thalipathiri chooranam, aqueous, silver nitrate and petroleum ether extract of siddha medicine were carried out to analyse the presence of alkaloid, flavanoid, phenol, terpenoid, saponin, reducing sugar, tannin, steroid and glycosides. Antioxidation potential of the Thalipathiri chooranam, aqueous, silver nitrate and petroleum ether extract of siddha medicine via. Nitric oxide radical scavenging and reducing power activity were carried out using standard procedure. *Thalipathiri* chooranam, aqueous, silver nitrate and petroleum ether extracts were tested for antibacterial activity against selected human pathogens viz. *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae and Proteus vulgaris* to create awareness about the significance of Siddha medicinal practices.

Result: *Thalipathiri* chooranam is a polyherbal formulation prepared from 15 different herbs being used for the treatment of *kapha (in Tamil)* diseases. The antibacterial assay revealed that the extracts corroborate good inhibitory activity against all test pathogens. The unexplored area of *Thalipathiri* chooranam towards their antioxidation effect in aqueous, silver nitrate and petroleum ether extracts indicated promising antioxidant activities in concentration dependent manner.

Conclusion: The present investigation concluded that the Siddha herbal preparations of chooranam have great potential as antioxidant and antimicrobial agent against many enteric pathogens. Thus these herbal preparations can be used to control or prevent the enteric bacterial infection.

Keywords: Polyherb, Siddha, *Thalipathiri* Chooranam, Antibacterial, Disc diffusion, Kalanchi, Antioxidation, Samoolam.

INTRODUCTION

Numerous sorts 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 [1]. 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 [2]. Many commercially proven drugs used in modern medicine were initially used in the crude form in traditional or folk healing practices or for other purposes that suggested potentially useful biological activity [3].

Microorganisms are the causative agents of almost all kinds of acute and chronic diseases. Plant based antibacterial 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 antibacterial. 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 [4]. 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 *Thalipathiri* Chooranam for antibacterial activity against selected human pathogens. The powder form of this Siddha Chooranam is used to treat cough and a wide range of *kapha* disease. Analyzing the phytochemicals and evaluating the antimicrobial 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. *Thalipathiri* Chooranam, aqueous, silver nitrate and petroleum ether extracts were screened for the presence of phytochemicals, antioxidant and antibacterial activity against selected human pathogens.

METHODS

Collection of plant materials

The plant materials were collected from unpolluted rural areas of India and ingredients were procured from commercial Siddha raw drug store was authenticated and prepared by my uncle (Siddha traditional practitioner). All the ingredients were shade dried, powdered and stored in porcelain pots. The Siddha formulation were prepared as prescribed in the written scripts, books and palm leaf parchments of my grandpa and forefathers - traditional vadiyars (Table 1). They are designated as Chooranam since they are comprised of multiple herbs.

Preparation of extract

All the dried herbs were finely powdered and the fresh leaves were triturated in household mortar and pestle without adding water. The powdered herbs were weighed (Kalanchi). The sampling Chooranam was subjected to maceration using different solvents aqueous, silver nitrate, and petroleum ether for 48 hrs. The extracts were filtered and evaporated to dryness and kept for further studies.

Phytochemical analysis of *Thalipathiri* Chooranam

The phytochemical analysis of *Thalipathiri* Chooranam, aqueous, silver nitrate and petroleum ether extract of Siddha medicine were carried

out to analyze the presence of alkaloid, flavanoid, phenol, terpenoid, saponin, reducing sugar, tannin, steroid, and glycosides [5,6].

Antioxidation assay

Nitric oxide radical scavenging

Nitric oxide radical scavenging capacity of *Thalipathiri* Chooranam and extracts with 0.1 ml of sodium nitroprusside (10 Mm) in phosphate buffer (0.2 M, pH 7.8) was mixed with different concentration was incubated at room temperature for 15 minutes. After incubation, 0.2 ml of Griess reagent was added. The absorbance of the reaction mixture was read at 546 nm against the blank. All the readings were taken in triplicate, and gallic acid was used as a standard [7]. The percentage of inhibition was calculated by the following equation:

$$\% \text{ Nitric oxide radical scavenging capacity} = ([A_0 - A_1]/A_0) \times 100$$

Reducing power activity

The reducing power activity of *Thalipathiri* Chooranam was determined by spectrophotometric method [8]. The extract was mixed with 2.5 ml of 0.2 M potassium phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes was then rapidly cooled, mixed with 2.5 ml of trichloroacetic acid and centrifuged at 5000 rpm for 3 minutes. An aliquot of the supernatant was diluted with distilled water, and 0.5 ml of 1% ferric chloride was added and allowed to stand for 10 minutes. The absorbance was read spectrophotometrically at 700 nm. Increased absorbance indicates increased reducing power. Vitamin C was used as positive control.

Antibacterial activity

Extract preparation

The sterile disks were soaked in *Thalipathiri* Chooranam, aqueous, silver nitrate and petroleum ether extracts of 100 g powder in 200 ml solvent for 12 hrs. The extracts were filtered using Whatman filter paper (125 mm) [9].

Growth and maintenance of test microorganism for antibacterial studies

Bacterial cultures of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Proteus vulgaris* were obtained from medical college were 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 for 24 hrs as overnight cultures.

Disc diffusion method

The antibacterial assay of *Thalipathiri* Chooranam and extracts was performed by disk diffusion method [10]. The NA media (20 ml) was poured into sterilized petridishes and left to solidify at room temperature. The overnight bacterial cultures have been spread plated on these petridishes using sterile L-rod. The filter paper discs were placed equidistantly on inoculated media and diffusion of the solution was allowed to occur for 30 minutes at room temperature. Plates were incubated at 37°C for 24 hrs. The average zone of inhibition was recorded. The diameters of the inhibition zones were measured in mm.

RESULTS AND DISCUSSIONS

Qualitative phytochemical analysis

The qualitative phytochemical analysis of *Thalipathiri* Chooranam revealed the presence of alkaloid, flavonoid, phenol, terpenoid, reducing sugar and tannin whereas, the absence of saponin, steroid, and glycosides. On the other hand, the aqueous extract revealed the presence of alkaloid, phenol, and terpenoid whereas, the absence of flavonoid, saponin, reducing sugar, tannin, steroid, and glycosides. The silver nitrate extract of the Chooranam revealed the presence of alkaloid, phenol and reducing sugar whereas, the absence of flavonoid, terpenoid, saponin, tannin, steroid, and glycosides. The petroleum ether extract revealed the presence of alkaloid, flavonoid, and tannin whereas, the absence of phenol, terpenoid, saponin, reducing sugar, steroid and glycosides. However, saponin, steroid and glycosides constituents were not observed in *Thalipathiri* Chooranam and its extracts whereas, maximum constituent of alkaloid is observed in *Thalipathiri* Chooranam and its extracts (Table 2).

Nitric oxide radical scavenging of *Thalipathiri* Chooranam

Nitric oxide injuries take place for the most part through peroxynitrite route because peroxynitrite can directly oxidize low-density lipoproteins, resulting in irreversible damage to the cell membrane. Inhibition increased with increasing concentration of the extract, the present investigation revealed that the *Thalipathiri* Chooranam and extracts showed nitric oxide scavenging activity [8]. The nitric oxide radical scavenging of *Thalipathiri* Chooranam, aqueous, silver nitrate and petroleum ether extracts increased gradually in concentration dependent manner. In general, among the medicine and extracts maximum reduction was noticed in *Thalipathiri* Chooranam 79.69±1.55 (100 µl) whereas, minimum reduction was noticed in silver nitrate extract 61±1.69 (100 µl). The tremendous result was observed in *Thalipathiri* Chooranam with scavenging ranges 79.69±1.55 at 100 µl than the extracts compared with 78.9±1.70 at 100 µl for gallic acid, which served as positive control. The increasing evidence suggests that the nitric oxide and its derivatives produce activated phagocytes may have a genotoxic effect and may contribute in the multistage carcinogenesis process [11]. The antioxidative defense systems and production of these reactive species in a healthy organism is approximately balanced. Antioxidant agents of natural origin have attracted special interest because they can protect the human body from free radicals (Table 3) [12].

Reducing power activity

The reducing power assay exhibited the presence of antioxidants in the extract, which resulted in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. The maximum reducing property was found at 100 µl (Table 4). The reducing power activity of *Thalipathiri* Chooranam, aqueous,

Table 1: Composition of *Thalipathiri* Chooranam

S. no.	Siddha name	Scientific name	Quantity
1	Athimathuram	*Glycyrrhiza glabra*	5 Kalanchi
2	Jathikkai	*Myristica fragrans*	3 Kalanchi
3	Chukku	*Zingiber officinale*	3 Kalanchi
4	Milagu	*Piper nigrum*	3 Kalanchi
5	Thippili	*Piper longum*	2 Kalanchi
6	Seeragam Samoolam	*Cuminum cyminum*	1 Kalanchi
7	Karumseeragam	*Nigella sativa*	3 Kalanchi
8	Lavangapattai	*Cinnamomum tamala*	3 Kalanchi
9	Kostam	*Saussurea lapa*	3 Kalanchi
10	Kadukkai	*Terminalia chebula*	3 Kalanchi
11	Thandrikkai	*Terminalia belerica*	3 Kalanchi
12	Parangipattai	*Smilax china*	2 Kalanchi
13	Vasambu	*Acorus calamus*	3 Kalanchi
14	Yanai Thippili	*Balanophora fungosa*	3 Kalanchi
15	Thaaleesapathiri	*Taxus beccata*	3 Kalanchi

Table 2: Qualitative phytochemical analysis of *Thalipathiri* Chooranam

Test	*Thalipathiri* Chooranam	Aqueous extract	Silver nitrate extract	Petroleum ether extract
Alkaloid	+	+	+	+
Flavanoid	+	–	–	+
Phenol	+	+	+	–
Terpenoid	+	+	–	–
Saponin	–	–	–	–
Reducing sugar	+	–	+	–
Tannin	+	–	–	+
Steroid	–	–	–	–
Glycosides	–	–	–	–

Table 3: Nitric oxide radical scavenging of *Thalipathiri* Chooranam

Concentration of medicine	Paavu Chooranam	Aqueous extract	Silver nitrate extract	Petroleum ether extract	Gallic acid
25 µl	37.11±0.97	22±2.14	14±1.10	13±1.50	30.2±1.50
50 µl	53.39±1.35	43±3.00	33±1.46	46±1.80	43.5±2.40
75 µl	69.00±1.11	57±2.30	54±1.20	61±0.56	63.2±1.90
100 µl	79.69±1.55	69±1.95	61±1.69	75±0.48	78.9±1.70

Table 4: Reducing power activity of *Thalipathiri* Chooranam

Concentration of medicine	Thalipathiri Chooranam	Aqueous extract	Silver nitrate extract	Petroleum ether extract	Vitamin C
25 µl	51.24±1.47	25.2±1.63	31.20±0.24	34.4±1.5	32.08±0.89
50 µl	64.22±0.71	36.3±0.86	35.1±1.08	43.30±1.2	47.05±1.06
75 µl	72.91±1.39	43.98±1.69	47.0±0.28	53.5±1.5	66.25±0.79
100 µl	83.29±1.05	65.12±0.83	51.21±0.20	58.7±1.05	81.69±1.08

silver nitrate, and petroleum ether extracts increased gradually in concentration dependent manner. In general, among the medicine and extracts maximum reduction was noticed in *Thalipathiri* Chooranam 83.29±1.05 (100 µl) whereas, minimum reduction was noticed in silver nitrate extract 51.21±0.20 (100 µl) (Table 4). The phenolic antioxidants usually scavenge free radicals by an electron transfer mechanism [13]. The reducing power capacity of extract may serve as a significant indicator of its potential antioxidant activity.

Antibacterial activity of *Thalipathiri* Chooranam

Antibacterial activity revealed that the tested extracts possess potential antibacterial activity against *S. aureus*, *P. aeruginosa*, *K. pneumoniae* and *P. vulgaris*. The diameters of the inhibition zones against all the tested bacteria were measured in mm. The disc diffusion method of *Thalipathiri* Chooranam showed significant activity against *S. aureus*, *P. aeruginosa*, *K. pneumoniae* and *P. vulgaris* around 14 mm. The highest antibacterial activity of 14 mm was observed in *S. aureus,* and least activity was recorded in *P. vulgaris* of 1-mm. The range of the zone of inhibition by *Thalipathiri* Chooranam against the human pathogens reported the maximum zone formation of 14 mm against *S. aureus* whereas, the minimum of 7 mm against *K. pneumonia*. On the other hand, the antibacterial activity of aqueous extract showed maximum activity against *P. aeruginosa* of 6 mm and least activity was observed in *S. aureus* of 4 mm. Inhibitory activity of silver nitrate showed minimum activity against *P. vulgaris* of 1-mm whereas, the maximum activity of 4 mm against *P. aeruginosa*. The petroleum ether extracts revealed the maximum zone formation of 6 mm against *S. aureus* whereas, the minimum of 3 mm against *K. pneumonia.* The obtained results elucidated that the activity was higher against Gram-positive strains than Gram-negative pathogens. On comparison of both the extracts the activity against pathogens showed similar results with effective and significant inhibitory action. In general zone formation was not observed in *K. pneumonia* of silver nitrate extract (Table 5).

Antibiotics provide the main basis for the therapy of bacterial infections. However, the high genetic variability of bacteria enables them to evade rapidly the action of antibiotics by developing antibiotic resistance. Thus, there has been a continuing search for new and more potent antibiotics [14]. The exploitation of plants for the management of plant diseases 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 *Thalipathiri* 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. 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

Table 5: Antibacterial activity of *Thalipathiri* Chooranam

Inhibition zone in diameter (mm)				
Siddha Chooranam & Extracts	**Pa**	**Sa**	**Pv**	**Kp**
Chooranam	12 mm	14 mm	9 mm	7 mm
Aqueous	6 mm	4 mm	5 mm	5 mm
Silver nitrate	4 mm	2 mm	1 mm	-
Petroleum ether	5 mm	6 mm	4 mm	3 mm
Amikacin	7 mm	8 mm	10 mm	6 mm

Pa: P. aeruginosa, Sa: S. aureus, Pv: Proteus vulgaris, Kp: K. pneumonia

by this compound [15]. Alkaloids can be used for treating cough has anti-tumor, analgesic property, and steroids contains anti-inflammatory activity.

CONCLUSION

The present work was a basic approach to find out the antioxidant and antimicrobial activity of Siddha medicine. Further works on the purification of individual groups of bioactive components might be able to reveal the exact potential of the chooranam to inhibit several pathogenic microbes. Our findings suggest that, Siddha herbal preparations have great potential as antioxidant and antimicrobial agent against many enteric pathogens. Thus these herbal preparations can be used to control or prevent the enteric bacterial infection.

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