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Research Article

Antioxidant Potential and Antibacterial Activity of Siddha Chooranam Prescribed to Cure Wound by the Traditional Siddha Medicinal Practitioner of Kanyakumari District, India

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Abstract

The polyherbal formulation of the Siddha chooranam comprised of 15 traditionally used herbs explore for the treatment of wound. The present investigation was mainly focused on the phytochemical, antioxidant potential and antimicrobial activities. The phytochemicals constituents in the herbal formulation revealed positive response of significant secondary metabolites. The unexplored area of chooranam towards their antioxidation effect in aqueous, chloroform and ethanol extracts indicated promising antioxidant activities of the crude extract.

Keywords: Polyherbal Formulation; Antioxidation; Qualitative Analysis; Chooranam; Kalanchi; Samoolam

Introduction

India has an eminent wealth of therapeutic agents for various ailments and diseases in our traditional system of medicine. Siddha Medicine is one of the oldest medical systems known to mankind. According to Siddha medicine system, diet and lifestyle play a major role not only in health but also in curing diseases. The formulations and treatment of Siddha medicine emphasizes that medical treatment must be oriented not merely to disease but also in view of the patient his environment, sex, age, habits, mental frame, diet, physical conditions etc. Phytochemicals with adequate antibacterial efficacy will be used for the treatment of the bacterial infections [1]. Antibiotics are the products of microbes that are powerful medicines fight against bacterial infections in dilute solution, inhibit or kill other organisms. Antimicrobial agents include antibiotics and synthetic compounds that have the same effect. Naturally occurring antibiotics may be modified to give semi-synthetic derivatives differ from their ferment compound as pharmacological properties. Those agents that kill bacteria are said to be bactericidal was reversible upon removal of the drug [2].

Primary metabolites such as carbohydrates, amino acids and fatty acids are directly useful for plants themselves. These are the metabolic intermediates of anabolic and catabolic pathways, which occur in plants, have the same metabolic functions. Plants also contain large variety of substances named secondary metabolites with no apparent direct metabolic functions [3]. All plants contain these compounds, which was once considered as being waste products but now generally accepted as defensive compounds against pathogens like bacteria, virus and fungus [4,5]. Traditional medicine is widespread and plants still presents a large source of natural antioxidants that might serve as leads for the development of novel drugs. Several anti-inflammatory, digestive, anti-necrotic, neuroprotective and hepatoprotective drugs have recently been shown to have an antioxidant or anti-radical scavenging mechanism as part of their activity [6].

Materials and Methods

The present investigation was aimed to assess the phytochemical constituents, antioxidant potential and antibacterial activity of the Siddha Chooranam used to cure wound prescribed by the Siddha Medicinal Practitioner.

Preparation of formulation

The ingredients were procured from commercial Siddha raw drug store was authenticated and prepared by the Traditional Siddha Medicinal Practitioner. The ingredients were shade dried, powdered and mixed thoroughly in same proportion was sieved and preserved in porcelain pots.

Analysis of antibacterial activity

Antibacterial activity was conceded out for the Siddha Chooranam used to cure Wound was tested against 4 bacterial pathogens.500g of Chooranam was bought from the Siddha Practitioner for the preparation of different solvent extracts was selected for the antibacterial activity test using different solvents via etha-

nol, aqueous and chloroform. Chooranam is prepared by the combination of medicinal plants in the form of Kalanchi.

Preliminary phytochemical analysis

Preliminary phytochemical screening of the crude extracts was determined by following the standard procedure [7].

Antioxidant activity

Antioxidant activity of the extract was analysed by following hydroxyl radical scavenging and reducing power activity. The absorbance of the resultant solution was measured using spectrophotometer (ELICO SL177).

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of extract was measured according to the method [8]. One milliliter of the final reaction solution consisted of aliquots (25µl, 50µl, 75µl and 100µl) of various concentrations of the ethanolic, aqueous and chloroform extracts, 1mM $FeCl_3$, 1mM EDTA, 20mM H_2O_2, 1mM L-ascorbic acid and 30mM De-oxyribose in potassium phosphate buffer (pH 7.4). The reaction mixture was incubated for 1 hour at 37°C and further heated in a boiling water bath for 15 minutes after addition of 1ml of 2.8% (w/v) trichloroacetic acid and 1ml of 1% (w/v) 2- thiobarbituric acid was measured at 532nm against a blank containing phosphate buffer.

Reducing power activity

Reducing power activity of the extract was determined by the method [9]. Different concentrations of extracts (25µl, 50µl, 75µl and 100µl) of ethanolic, aqueous and chloroform extracts respectively were mixed with 2.5ml of phosphate buffer (200mM, pH 6.6) and 2.5ml of 1% potassium ferricyanide. The mixtures were incubated for 20 minutes at 50°C. After incubation, 2.5ml of 10% trichloroacetic acid was added to the mixtures, followed by centrifugation at 650×g for 10minutes. The upper layer (5ml) was mixed with 5ml of distilled water and 1ml of 0.1% ferric chloride and the absorbance of the resultant solution were measured at 700nm using spectrophotometer (ELICO SL177).

Preparation of solvent extracts

The solvent extract of ethanol, aqueous and chloroform of 5g Chooranam were completely dissolved in 5 ml of 0.5% Tween 80 and preserved at 5°C in airtight bottles until further use [10]. All the extracts were subjected to antibacterial activity assay.

Antimicrobial susceptibility test

Four human pathogens selected for the antibacterial assay are *Pseudomonas aeruginosa, Staphylococcus aureus, Proteus vulgaris* and *Klebsiella pneumonia*. Muller - Hinton agar is used as the nutrient medium for the selected bacterial strains. General methodology was followed for the preparation and sterilization of agar medium. Disks of 4 mm diameter were cut from Whatman No.4 filter paper for inhibitory study. The discs were taken in culture tubes and sterilized using autoclave at 121°C under 15 lbs pressure for 15 minutes. Disc diffusion method was used to screen the antimicrobial activity. The empty sterile disc was dipped in the respective extracts and dried in room temperature placed on the inoculated agar medium in petriplates with the sterilized forceps [11]. Then the plates were incubated at 37° C for 24 hours. The antibacterial activity of Chooranam was observed through zone of inhibition around the disc was measured in millimeter and tabulated.

Results and Discussions

The Siddha Chooranam prescribed to cure wound was mixed with aqueous, ethanol and chloroform extract. The qualitative, quantitative, antioxidant and antimicrobial activity was done. The clinical isolates of *Proteus vulgaris, Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumonia* were used for the antibacterial assay. The obtained datas were tabulated and discussed.

Qualitative analysis

Green plants synthesis and preserve variety of bio-chemical products are extracted and used as chemical feed stocks or as raw material for various scientific investigations. The World Health Organization (WHO) estimated that 80% of the World population depends on traditional system of medicine. Preliminary phytochemical screening of different metabolites via steroids, alkaloids, sugars, phenolic compounds, flavonoids, saponins, tannins, anthroquinone and aminoacids in aqueous, ethanol and chloroform extracts of Siddha Chooranam revealed the presence or absence of different metabolites.

Qualitative analysis of siddha chooranam and extracts

Phytochemical analysis of the Siddha Chooranam revealed the presence of alkaloid, phenol and tannin whereas, the absence of steroids, sugars, flavonoids, saponins, anthroquinone and aminoacids. Aqueous extract revealed the presence of phenolic constituents alone whereas, the absence of steroids, alkaloids, sugars, flavonoids, saponins, tannins, anthroquinone and aminoacids. The ethanolic extract revealed the presence of tannin whereas, the absence of steroids, alkaloids, sugars, phenolic compounds, flavonoids, saponins, anthroquinone and aminoacids. The phytochemical analysis of chloroform extract revealed the presence of phenol and flavanoid constituents whereas, the absence of steroids, alkaloids, sugars, saponins, tannins, anthroquinone and aminoacids (Table 1). Alkaloid can be used for treating wound and plant phenolics are a major group of compounds that act as a primary antioxidant [12].

Quantitative Analysis

The major phytochemical constituents of Siddha Chooranam were quantified after the addition of chemical ingredients, measured at 700nm using ELICO Spectrophotometer. The quantitative phytochemical analysis reported the presence of Alkaloid 0.229 ±

S. No	Phyto-chemicals	Observations			
		Chooranam	Aqueous	Ethanol	Chloroform
1	Steroids	-	-	-	-
2	Alkaloids	+	-	-	-
3	Sugar	-	-	-	-
4	Phenol	+	+	-	+
5	Flavonoids	-	-	-	+
6	Saponins	-	-	-	-
7	Tannins	+	-	+	-
8	Anthroqui-none	-	-	-	-
9	Aminoacid	-	-	-	-

Table 1: Phytochemical Screening of Siddha Chooranam and Extracts.

+: Presence; -: Absence.

0.076 µg/g; Phenol 0.897 ± 0.299 µg/g and Tannin 2.352 ± 0.784 µg/g constituents (Table 2).

S. No	Phytochemical Constituents	Result
1	Alkaloid	0.229 ± 0.076µg/g
2	Phenol	0.897 ± 0.299 µg/g
3	Tannin	0.605 ± 0.201 µg/g

Table 2: Quantitative Analysis of Siddha Chooranam used to cure wound.

Antioxidation assay

Antioxidation assay of hydroxyl radical scavenging activity and reducing power activity of the Siddha chooranam, aqueous, ethanol and chloroform extract after the addition of chemical ingredients were measured at 523 nm using ELICO Spectrophotometer.

Hydroxyl radical scavenging activity of siddha chooranam and extracts

Hydroxyl radical scavenging is an extremely reactive free radical formed in biological system. Hydroxyl radical scavenging of the Siddha Chooranam revealed, minimum inhibition 27.08% at low concentration (25µl) whereas, maximum inhibition 85.08% at high concentration (100µl). On the other hand, aqueous extract showed minimum inhibition 1.48% at low concentration (25µl) whereas, maximum inhibition 5.90% at high concentration (100µl). The ethanolic extract showed minimum inhibition 0.38% at low concentration (25µl) whereas, maximum inhibition 3.04% at high concentration (100µl). The chloroform extract showed minimum inhibition 32.62% at low concentration 25µl whereas, maximum inhibition 50.46% at high concentration (100µl) (Figure 1).

Figure 1: Hydroxyl Radical Scavenging of Siddha Chooranam and Extracts.

Reducing power activity of siddha chooranam and extracts

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 reducing power of Chooranam increased gradually in concentration dependent manner, Siddha chooranam revealed, minimum inhibition 39.41% at low concentration (25µl) whereas, maximum inhibition 99.50% at high concentration (100µl). On the other hand, aqueous extract showed minimum inhibition 0.38% at low concentration (25µl) whereas, maximum inhibition 7.14% at high concentration (100µl). The ethanolic extract showed minimum inhibition 0.53% at low concentration (25µl) whereas, maximum inhibition 3.00% at high concentration (100µl). The chloroform extract showed minimum inhibition 37.05% at low concentration 25µl whereas, maximum inhibition 45.22% at high concentration (100µl). The standard antioxidant Vitamin – C showed minimum inhibition 53.71% at low concentration 25µl whereas, maximum inhibition 98.39% at high concentration (100µl) (Figure 2). Exogeneous chemical and endogenous metabolic processes in the human body or food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing bio-molecules, 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 [13].

Figure 2: Reducing Power Activity of Siddha Chooranam and Extracts.

Antibacterial activity

The clinical isolates of *Proteus vulgaris, Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumonia* were used for the antibacterial assay. The antimicrobial activity of the selected microorganisms was determined using Disk diffusion method. The extract loaded disks were measured after 24 h of incubation. The antibacterial activity of Siddha medicine prescribed for Wound (Chooranam) showed both positive and negative activity. Chooranam showed the maximum zone of inhibition (28mm) against *Pseudomonas aeruginosa*, whereas, *Staphylococcus aureus* and *Proteus vulgaris* showed the maximum zone of inhibition (16mm) and minimum zone of inhibition (5mm) against *Klebsiella pneumonia.* The antibacterial activity of ethanolic extract of the chooranam showed the maximum zone of inhibition (13mm) against *Proteus vulgaris* Whereas, *Staphylococcus aureus* showed the maximum zone of inhibition (11mm) and minimum zone of inhibition (2mm) against *Pseudomonas aeruginosa* and *Klebsiella pneumonia.* The antibacterial activity of aqueous extract of the chooranam showed the maximum zone of inhibition (13mm) against *Pseudomonas aeruginosa* minimum zone of inhibition (2mm) against *Staphylococcus aureus* and *Proteus vulgaris*. On the other hand, aqueous extract of Chooranam fail to inhibit the growth of the bacteria *Klebsiella pneumonia.* The antibacterial activity of chloroform extract of the chooranam showed the maximum zone of inhibition (4mm) against *Pseudomonas aeruginosa* (3mm) against *Staphylococcus aureus* and minimum zone of inhibition (2mm) against Proteus vulgaris and *Klebsiella pneumonia* (Table 3).

Siddha Medicine and Extracts	Inhibition Zone in diameter (mm)			
	Pa	Sa	Pv	Kp
Chooranam	28mm	16mm	16mm	5mm
Ethanol	2mm	11mm	13mm	2mm
Aqueous	13mm	2mm	2mm	-
Chloroform	4mm	3mm	2mm	2mm
Amikacin	37mm	18mm	19mm	8mm

Table 3: Antibacterial activity of Siddha Chooranam and Extracts.

Pa: *Pseudomonas aeruginosa*; Sa: *Staphylococcus aureus*; Pv: *Proteus vulgaris;* Kp: *Klebsiella pneumonia.*

Conclusion

The phytochemical screening of the Siddha Chooranam showed the presence of secondary metabolites like alkaloid, phenols and tannin constituents. The assessment of phytochemical constituents, antioxidant and anti-bacterial activity of the Siddha Chooranam prescribed to cure wound revealed the importance to create awareness about the significance of unexplored medicinal practice and value of its perpetuation. The present work carried was a basic approach to find out the antimicrobial activity in 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.

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