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Research Article

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Preliminary phytochemical and antioxidant screening of Athimadhura chooranam

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Abstract

Scientific documentation of traditional system of medicine is increasing and need for preparing it for Siddha formulation. Siddha classical texts have numerous polyherbal formulations, used for the treatment of various diseases. *Athimadhura chooranam* is a polyherbal formulation consisting 8 traditionally used herbs. Since there is no information about antioxidant and phytochemical evaluations of Athimadhura chooranam. Hence the present study was screening of phytochemical and *in vitro* evaluation of antioxidant activity of Athimadhura chooranam. The aqueous-methanol extract of Athimadhura chooranam was screened for phytochemicals which indicated the presence of flavonoid, glycosides, triterpenes, coumarins, phenols, cardiac glycosides, saponins and betacyanin. For antioxidant activity, the methanol extract of Athimadhuram chooranam will have to contain as much quantity of antioxidants compounds as equivalents of ascorbic acid to effectively reduce the oxidant in the reaction matrix. The result indicated promising antioxidant activity of crude extract and needs further exploration for their effective use in both modern and traditional system of medicines.

Keywords: Siddha medicine, Athimadhura chooranam, phytochemical, antioxidant.

Introduction

Medicinal plants have been an important source of medicine for thousands of years. According to the World Health Organization (WHO) more than 80% of the world's population.

Realize on traditional medicine for their primary health care needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment (Sindhu *et al*., 2013). Siddha medicine is considered as the oldest medical system known to mankind. There are numerous plants and traditional formulations available in Siddha system of medicine to treat various human ailments because they contain the components of therapeutic value (Mutheeswaran *et al*., 2014). Athimadhura chooranam (AMC) is a combination of Athimadhuram (*Glycirrhiza glabra*), Elam (*Elettaria cardamomum*), Elavangapattai (*Syzygium aromaticum*), Senbaga Mokku (*Michelia champaca*), Kottam (*Costus speciosus)*, Sukku (*Zingiber officinale*), Nar Seeragam (*Cuminum cyminum*), Korai Kizhangu (*Cyperus rotundus*) and Sugar.

The active principles of many drugs found in plants are secondary metabolites. Therefore, basic phytochemical investigation of these extracts for their major phytoconstituents is also vital (Ghani, 1990; Dobelis, 1993). Free radicals are chemical species which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. Free radicals are continuously produced in the human body, as they are essential for energy

supply, detoxification, chemical signaling and immune function (Gulcin, 2005). An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage and oxidative stress is the main cause of several diseases: cancer, cataracts, age related diseases and Parkinson's disease.

Antioxidants reduce the oxidative stress in cells and are therefore useful in the treatment of many human diseases, including cancer, cardiovascular diseases and inflammatory diseases. This activity is due to the ability of antioxidants to reduce oxidative stress by neutralizing or scavenging of reactive species by hydrogen donation (Lindenschmidt *et al*., 1986).

Hence, certain amounts of exogenous antioxidants are constantly required to maintain an adequate level of antioxidants in order to balance the ROS. Recently, many epidemiological studies have suggested that the consumption of natural antioxidants such as polyphenol-rich food, fresh fruits, vegetables, or teas have protective effects against the aforesaid diseases and this protection has been partly ascribed to the presence of several components, such as vitamins, flavonoids, and other phenolic compounds (Klimczak *et al*., 2007).

Presently, much attention has been focused on the use of natural antioxidants to protect the human body especially brain tissues from the oxidative damage caused by free radicals. In last two decades, several medicinal plants have shown such effectiveness through the traditional methods of psychoneuropharmacology (Dhawan, 1995). Therefore, the aim of the study was screening of phytochemical and *in vitro* evaluation of antioxidant activity of Athimadhura chooranam.

Materials and Methods

Drug collection, Identification and Authentication

All the ingredients were obtained from Country drug shop, Ramasamychetti, Parrys Chennai, Tamilnadu, India. All the raw drugs were identified and authenticated at Central Research Institute (CRI), Arumbakkam, Chennai-106

Ingredients of chooranam

The medicinal plants Athimadhuram (*Glycirrhiza glabra*), Elam (*Elettaria cardamomum*), Elavangapattai (*Syzygium aromaticum*), Senbaga Mokku (*Michelia champaca*), Kottam (*Costus speciosus)*, Sukku (*Zingiber officinale*), Nar Seeragam (*Cuminum cyminum*), Korai Kizhangu (*Cyperus rotundus*) and Sugar composed in Athimadhuram chooranam were prepared.

Purification of raw drugs

Raw drugs are purified a mentioned in Sikicharathna Deepam Sarakku Suthi Muraigal.

Preparation of chooranam

The purified above first 8 raw drugs are made into fine powder as mentioned in the literature and finally sugar added.

Phytochemical analysis

The choorana powder was extracted by hot percolation method using Soxhlet apparatus. The solvents used were methanol and water. About 40 gm of powder was extracted with 200 ml of methanol/water (6:4). The extract was concentrated to dryness under controlled temperature 40-50°C. The extract was preserved in refrigerator till further use. For the tannins experiment, few drops of ferric chloride solution was added with extract and noted the colour change.

Phytochemical analysis

Phytochemical profile of chooranam extract was analyzed as per the methodology of Harborne (Harborne, 1973). For alkaloid test, 1 ml of sample was added with 1 ml of diluted acetic acid and few drops of Dragendorff's reagent and noted the precipitate. Coumarin assessed by 0.5 ml of extract was taken in a test tube with 1 ml NaOH and shaken well and noted the colour change. Presence of saponins was examined by taking the extract with water and shaken hardly and noted for froth. Glycosides were analyzed by hydrolysed the drug with concentrated HCl, and 2 ml of filtered hydrolysate, 3 ml of choloroform is added and shaken, choloroform layer is separated and 10% ammomia solution is added to it and noted for pink colour. For flavonoids test, 0.5 ml of extract was taken and 1 mg of magnesium turning and few drops of concentrated hydrochloric acid were added, boiled for 5 min and noted the colour change.

The phenol was assessed by 0.5 ml of extract was taken with 1 ml of alcoholic ferric chloride and noted for colour change. For cardial glycosides test, the extract treated with 2 ml glacial acetic acid containing a drop of FeCl3 and observed brown colour ring. For steroids test one ml of extract was taken and add few drops of concentrated sulphuric acid, shake well and kept away some time and noted for colour change.

To test the quinone, 0.5 ml of extract was added with 1 ml of sodium hydroxide (10%) and observed for colour change. For test the cyanin**,** 2 ml of the extract, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100^0C. Formation of bluish green colour indicates the presence of anthocyanin and of yellow colour indicates the presence of betacyanin. Terpenoids were analyzed by 5ml of extract was mixed in 2ml of chloroform, and concentrated sulphuric acid was carefully added to form a layer and observed A reddish brown colouration. To check the presence of sugars, extract was treated with

Fehlings solution A and B and boiled and noted for precipitation colour.

Antioxidant activity

Different concentrations of extract (125-2000µg/ml) were obtained with 3ml of reagent solution (0.6ml H_2SO_4, 28mM sodium phosphate and 4mM ammonium molybdate). The tube containing the reaction solutions were incubated at 95^0c for 90 minutes. The absorbance of the solution was measured at 695nm against blank after cooling to room temperature (Methanol 0.3ml) in the place of extract was used as blank. The antioxidant activity is expressed as number of gram equivalent of ascorbic acid.

Results and Discussion

Phytochemical screening of Athimadhuram chooranam

Phytochemical screening provides basic information about medicinal importance of a plant extract. In this study, evaluation for qualitative analysis of the chemical constituents of Athimadhuram chooranam extracts showed the presence of various secondary metabolites, flavonoid, glycosides, triterpenes, coumarins, phenols, cardiac glycosides, saponins and betacyanin. Alkaloids, steroids, carbohydrates, tannins, anthocyanin and quinines were not detected in aqueous-methanol extract (Table 1). Phytochemical screening indicated that the aqueous-methanol extract contained phenols and flavonoids, which are phenolic compounds. Plant phenolics are known to be antioxidants and free radical scavengers. The presence saponins compound has been shown to have immense significance as antihypercholesterol, hypotensive and cardiac depressant properties (Price *et al*., 1987). The cardiac glycosides have been used for over two centuries as stimulants in cases of cardiac failure (Cherian and Augusti, 1995).

Table 1: Phytochemical screening of aqueous-methanol extract from Athimadhuram chooranam

Phytocomponents	Solvent
Alkaloids	-
Flavonoids	+
Glycosides	+
Steroids	-
Carbohydrates	-
Triterepnoids	+
Coumarins	+
Phenols	+
Cardiac glycosides	+
Tannins	-
Saponins	+
Proteins	-
Anthocyanin	-
Betacyanin	+
Quinones	-

Antioxidant activity

Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer (Nooman et al., 2008). The antioxidant activity for the different concentrations of Athimadhuram chooranam was evaluated by using phosphomolybdate method. It determines the total antioxidant capacity. This assay is based on the reduction of Mo(VI) to Mo(V) in presence of the antioxidant compounds and the subsequent formation of a green phosphate/Mo(V) complex at acidic pH, which is measured at 695 nm. Total antioxidant capacity of Athimadhuram chooranam extracts, expressed as equivalents of ascorbic acid (µg/mL of extract), is shown in table 2 and Figures 1. The antioxidant capacity of athimadhuram chooranam extracts showed an increase in antioxidant capacity with an increase in dose.

The results indicate a concentration dependent total antioxidant capacity, it means that the methanol extract of Athimadhuram chooranam will have to contain as much quantity of antioxidants compounds as equivalents of ascorbic acid to effectively reduce the oxidant in the reaction matrix.

Table 2: Total antioxidant activity of Athimadhuram chooranam

Concentration	OD value(I)	OD value(II)	OD value(III)	Average OD	% of Inhibition
Control	0.1396	0.1396	0.1396	0.1396	
125	0.1001	0.1289	0.0989	0.1093	21.70
250	0.0996	0.0976	0.0920	0.0964	30.95
500	0.0548	0.0702	0.0585	0.0612	56.18
1000	0.0342	0.0396	0.0284	0.0341	75.60
2000	0.0246	0.0302	0.0261	0.0270	80.68

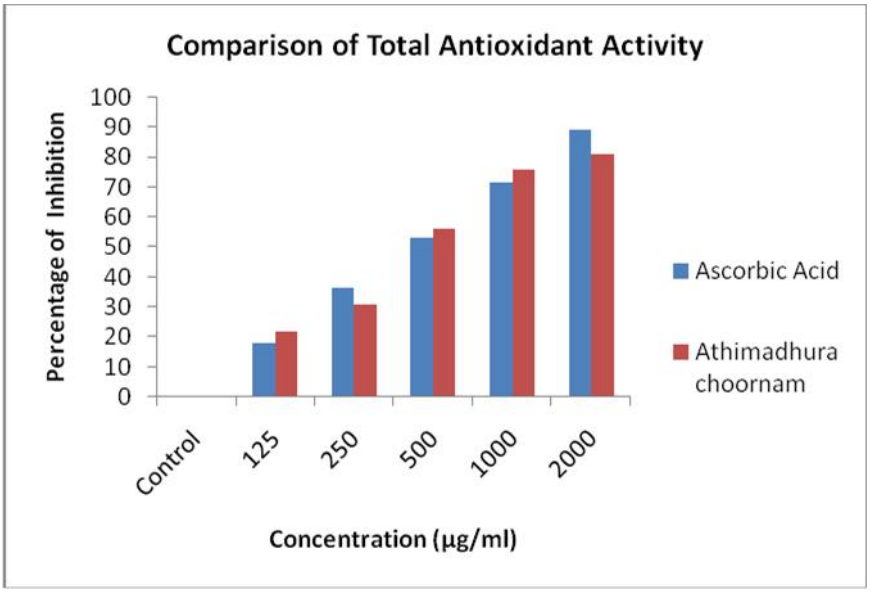

Fig 1: Total antioxidant activity of Athimadhuram chooranam

Antioxidant capacity of ascorbic acid has been used as a reference standard from which plant extracts with potential antioxidant activity are compared (Aderogba *et al*., 2005). This good antioxidant activity might be attributed to the presence of high amounts of polyphenols in these extracts

Conclusion

The present study indicates the presence flavonoid, glycosides, triterpenes, coumarins, phenols, cardiac glycosides, saponins and betacyanin during the phytochemical investigation. The Athimadhuram chooranam was used to evaluate the antioxidant potential using Phosphomolybdenum reduction assay method. The methanol extract of Athimadhuram chooranam showed higher phenolic content as well as flavonoid content and contributes to the higher antioxidant activity. Considering the results obtained it could be concluded that the plant contains essential phytochemical constituents and possess active antioxidant property. Further investigations may be carried out to find active component of the extract and to confirm the mechanism of action.

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