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RESEARCH ARTICLE

AN INVTRO STUDY ON ANTI-OBESITY, ANTIRADICAL ACTIVITIES OF SIDDHA MEDICINE NATHAI CHOORI CHOORANAM

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ABSTRACT

Traditional systems of medicine have been in trend for treating numerous ailments in many develop and development countries such as India, China, Japan and Korea, Tavian since age-old time. Siddha system of medicine is one of the ancient traditional system of India and practiced mostly in its southern part of India for treating different diseases including even chronic conditions. Nathai choori chooranam composed of five plant equal composition (Borreria hispida seed, Zingiber officinale Rhizome, Cassia auriculata flower, *And*dry fruit and Eleusine coracana seed). Plants offer us bioactive molecules those may serve as safer therapeutics to combat existing new world ageing related diseases, and obesity is the major concern among them. Pancreatic lipase inhibitors from plant sources may prove as promising side effects lacking anti-obesity therapeutics, present study was conceived with the objective of anti-lipase, antiradical and DNA cleavage protector activities of Nathai choori chooranam decoction. Phytochemical constituents were screened quantifications of total phenolics, tannins, flavonoids, were done by taking tannic acid, quercetin, as reference molecules. Antiradical activities were evaluated by using different free radicals (ABTS, lipid peroxidation, metal chelating, superoxide and nitric oxide scavenging activities). Also effect of Nathai choori chooranam decoction on DNA cleavage induced by H_2O_2 UV-photholysis. The antiradical activity of Nathai choori chooranam decoction was proved to be better than the standard ascorbic acid. Antilipolytic function of Nathai choori chooranam decoction was assessed using porcine pancreatic lipase (PPL; triacylglycerol lipase, EC 3.1.1.3) in an in vitro assay system with p-nitrophenyl palmitate (p-NPP) as a substrate. From the data of the results obtained, maximum percentage of lipase inhibition was shown by Nathai choori chooranam decoction (78.92 %) than standard drug. In addition, they showed a protective effect on DNA cleavage.IC50 value were calculated using the logarithmic regression of the dose-response curve after subtraction of both blank and inherent sample fluorescence values. In all cases, the coefficients of determination of the regression (R2) were greater than 0.95. IC50 are the means ± standard deviations of three determinations.

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INTRODUCTION

Obesity is having too much body fat which mean that weight of a person is larger than the weight that is to be for healthy living for his height. The increase of obesity is characterized by a chronic unevenness between energy intake and energy spending, and it is often attributed to changing lifestyles and insufficient dietary habits. Also, reduced energy spending is often related with a hereditary low basal metabolic rate, physical in activity, and low volume for fat oxidation.

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Epidemiological studies have shown a direct relation between the incidence of overweight/obesity and dietary fat consumption. Humans are frequently exposed to fat rich foods, which are usually associated with a high-energy intake. Thus, those foods with a high-energy and dietary fat content are considered to promote body fat storage and weight gain in human. Obesity is one of the major threats to global health in this century in both developed and developing countries (Moreno *et al*., 2003). More than 1.5 billion over weight adults and a minimum of 400 million clinically obese persons are being suffered by this health hazard. In the European Union, approximately 60% of adults and over 20% of school-age children are overweight or obese.

In England over a quarter of adults (26%) were reported to be obese in 2015. In 2009-2010, 35.7% of U.S. adults and almost 17% of youth were recorded as obese (Cynthia *et al*., 2015). Indians are also reported for obesity and its associated significances. Obesity leads to a key factor for health issues like hyper lipidemia, high blood pressure, type II diabetes, Cardiovascular disease reproductive and gastrointestinal cancers, gallstones, fatty liver disease, osteoarthritis and sleep apnea and also some form of cancer such as reproductive and gastrointestinal Cancers prostate cancer. Natural bioactive compounds especially from plant sources, including spices have been investigated for their characteristics and health effects. Plants are potential sources of natural bioactive compounds such as secondary metabolites and antioxidants. They absorb the sun light and produce high levels of oxygen and secondary metabolites by photosynthesis. Medicinal components produced are stored in plant leaves. Most of the secondary metabolites of herbs and spices are commercially important and find use in a number of pharmaceutical compounds. Flavonoids and phenolics acids are the most important groups of secondary metabolites and bioactive compounds in plants (Kim *et al*., 2003). They are also a kind of natural product and antioxidant substance capable of scavenging free superoxide radicals, anti-aging and reducing the risk of cancer (Bodeker, 2000).

MATERIALS AND METHODS

Preparation of extract

Nathai choori chooranam composed of five plant equal composition (*Borreria hispida* seed, *Zingiber officinale* Rhizome, *Cassia auriculata* flower, *And* dry fruit and *Eleusine coracana* seed).All the dried herbs were finely powdered and triturated in house hold mixer grinder without adding water. Then all the powdered herbs were weighed about 14.28g and mixed evenly. Aqueous decoction made into sterile distilled water in water both 100 ºC for 1h. The extracts were filtered and evaporated to dryness and kept for further studies.

Phytochemical analysis of Nathai choori chooranam

The aqueous decoction of Nathai choori chooranam were freshly prepared and various chemical constituents were analysed according to methods described by Allen and Harbone. The different chemical constituents tested for included tannins, saponin, glycosides, alkaloids, terpenoids, anthocynin, polyphenol and flavonoids.

Pancreatic lipase inhibitory activity

The lipase inhibition activity of plant extract was determined as per the method proposed by Kim *et al*. (2010). In this assay, the porcine pancreatic lipase activity was measured using p-nitrophenyl butyrate (NPB) as a substrate. Lipase solution (1 mg/mL) was prepared in a 0.1 mM potassium phosphate buffer (pH 6.0). To determine the lipase inhibitory activity, 1 ml of aqueous decoction of Nathai choori chooranam were pre-incubated with 1 ml of lipase for 10 min at 37°C. The reaction was then started by adding 0.1 mL NPB substrate. After incubation at 37°C for 15 min, the amount of p-nitrophenol released in the reaction was measured at 405 nm using a UV-Visible spectrophotometer and the percentage of inhibitory activity was calculated. ABTS (2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical scavenging assay of aqueous decoction of Nathai choori chooranam ABTS radical scavenging activity of aqueous decoction of Nathai choori chooranam were determined according to Re *et al*.1999. ABTS radical was freshly prepared by adding 5 ml of a 4.9 mM potassium per sulfate solution to 5 ml of a 14 mM ABTS solution and kept for 16 h in dark. This solution was diluted with distilled water to yield an absorbance of 0.70 at 734 nm and the same was used for the antioxidant assay. The final reaction mixture of standard group was made up to 1 ml with 950 µl of ABTS solution and 50 µl of Vit-C. Similarly, in the test group 1 ml reaction mixture comprised 950 µl of ABTS solution and 50 µl of the extract solutions. The reaction mixture was vortexed for 10 s and after 6 min absorbance was recorded at 734 nm against distilled water by using an ELICO (SL150) UV–Vis Spectrophotometer and compared with the control ABTS solution. Ascorbic acid was used as reference antioxidant compound.

Superoxide radical scavenging assay of aqueous decoction of Nathai choori chooranam

This assay was based on the capacity of the extract to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) (Beauchamp and Fridovich, 1971) in the presence of the riboflavin-light-NBT system, as described earlier Tripathi and Pandey (1999); Tripathi *et al*. 1998. Each 3 ml reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 2 µM riboflavin, 100 µM EDTA, NBT (75 µM) and different concentration of aqueous decoction of Nathai choori chooranam sample solution. It was kept in front of fluorescent light and absorbance was taken after 6 min at 560 nm by using an ELICO (SL150) UV–Vis Spectrophotometer. Identical tubes with reaction mixture were kept in the dark and served as blanks. The percentage inhibition of superoxide generation was measured by comparing the absorbance of the control and those of the reaction mixture containing test sample solution.

% Super oxide radical scavenging capacity= [(A0- A1)/A0] ×100

Where A0 was the absorbance of control and A1 was the absorbance of organic solvent extract or standard.

Inhibition of Lipid Peroxidation activity of aqueous decoction of Nathai choori chooranam

Lipid peroxidation induced by Fe_{2+}-ascarbate system in egg yolk by the method of Bishayee and Balasubramaniyam 1971, was estimated as thiobarbituric acid reacting substances (TBARS) by the method of Ohkawa *et al*.1977. The reaction mixture contained egg yolk 0.1 ml (25% w/v) in Tris-HCl buffer (20mM, pH 7.0); KCl (30mM); $FeSO_4$ $(NH_4)_2SO_4.7H_2O$ (0.06mM); and various concentrations of aqueous decoction of Nathai choori chooranamin a final volume of 0.5ml. The reaction mixture was incubated at 37^oC for 1 h. After the incubation period, 0.4ml was removed and treated with 0.2ml sodium dodecyl sulphate (SDS) (1.1%); 1.5 ml thiobarbituric acid (TBA) (0.8%); and 1.5 ml acetic acid (20%, pH 3.5). The total volume was made up to 4.0 ml with distilled water and then kept in a water bath at 95 to 100^oC for 1 h. After cooling, 1.0 ml of distilled water and 5.0 ml of n-butanol and pyridine mixture (15:1 v/v) were added to the reaction mixture, shaken vigorously and centrifuged at 4000 rpm for 10 min. The butanol-pyridine layer was removed and its absorbance at 532 nm (ELICO (SL150) UV–Vis Spectrophotometer) was

measured to quantify TBARS. Inhibition of lipid peroxidation was determined by comparing the optical density (OD) of treatments with that of the control. Ascorbic acid was used as standard. Inhibition of lipid peroxidation (%) by the extract was calculated according to 1- (E/C) X 100, where C is the absorbance value of the fully oxidized control and E is absorbance of the test sample ($Abs_{532+TBA} - Abs_{532\_TBA}$).

Metal chelating activity of aqueous decoction of Nathai choori chooranam

Metal chelating capacity of aqueous decoction of Nathai choori chooranam were measured according to the method described by Iihami *et al*. 2003. 1 mL of different concentrations of ethanolic extract was added to a 0.05ml of 2 mM ferric chloride solution. The reaction was initiated by the addition of 0.2 mL of 5 mM Ferrozine and the mixture was shaken vigorously. After 10 min, the absorbance of the solution was measured at 562 nm against blank. All readings were taken in triplicate and Vitamin C was used as the standard. The % inhibition of ferrozoine- Fe^{2+} complex was calculated by following equation.

% Inhibition of ferrozoine- Fe^{2+} complex = [(A0- A1)/A0] ×100

Where A0 was the absorbance of control and A1 was the absorbance of different solvent extract.

Nitric oxide radical scavenging activity of aqueous decoction of Nathai choori chooranam

Nitric radical scavenging capacity of aqueous decoction of Nathai choori chooranam were measured according to the method described by Olabinri *et al*.2010. 0.1ml of sodium nitroprusside (10mM) in phosphate buffer (0.2 M, pH 7.8) was mixed with different concentration of ethanolic extract and incubated at room temperature for 150 min. After incubation period, 0.2 mL of Griess reagent (1% Sulfanilamide, 2% Phosphoric acid and 0.1% N- (1- Naphthyl) ethylene diamine dihydrochloride) was added. The absorbance of the reaction mixture was read at 546nm against blank. All readings were taken in triplicate and Curcumin was used as the standard. The % inhibition was calculated by following equation.

% Nitric oxide radical scavenging capacity= [(A0- A1)/A0] ×100

Where A0 was the absorbance of control and A1 was the absorbance of different solvent extract.

RESULTS AND DISCUSSION

Phytochemical screening of aqueous decoction of Nathai choori 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 aqueous decoction of Nathai choori chooranam showed the presence of various secondary metabolites, alkaloid, saponins, flavonoid, tannins, polyphenols, anthraquinones and triterpenes. Cardiac glycosides were not detected in aqueous decoction (Table-1). Phytochemical screening indicated that the aqueous decoction extract contained tannins and flavonoids, which are phenolic compounds. Plant phenolics are known to be antioxidants and free radical scavengers.

Inhibition of pancreatic lipase activity of aqueous decoction of Nathai choori chooranam

In the present study, aqueous decoction of Nathai choori chooranam have been evaluated for lipid lowering activity through percentage inhibition of pancreatic lipase. Table-2 shows the results of pancreatic Lipase inhibition of the aqueous extract of selected medicinal plants at various concentrations. From the data of the results obtained, maximum percentage of lipase inhibition was shown by aqueous decoction of Nathai choori chooranam (78.92 %). The Orlistat standard has shown minimum activity (69.93 %) (Table-2). Pancreatic lipase inhibition is the most widely studied mechanism for the identification of potential anti-obesity agents. Only one blockbuster drug, Orlistat approved by FDA and available for the obesity treatment apart from the centrally acting anti-obesity drugs, is acting through the pancreatic lipase inhibition. Discovery of orlistat was done from the naturally occurring molecule lipstatin. The success of naturally occurring compounds for treatment of obesity has influenced the research for the identification of newer pancreatic lipase inhibitors that lack unpleasant side effects.

ABTS radical activity of aqueous decoction of Nathai choori chooranam

Aqueous decoction of Nathai choori chooranam exhibited a powerful scavenging activity for ABTS radical cations in a concentration dependent manner (Table-3), showing a direct role in catching free radicals. Maximum discoloration was observed with the aqueous decoction ranges from 27.89 to 82.63% at 100-400 µl/ml than Vitamin-C. This property may be credited to the presence of polyphenolics and flavones in the Nathai choori chooranam. Hagerman *et al*. (1998) have stated that the high molecular weight phenolics (tannins) have more abilities to quench free radicals (ABTS) and their effectiveness depends on the molecular weight, the number of aromatic rings and nature of hydroxyl group's substitution than the specific functional groups. Free radical (ABTS) scavenging activity of Nathai choori chooranam decoction might be due to the presence of high molecular weight phenolics such as catechin, and rutin derivatives. All the observations in different groups showed significant ($P < 0.01$) relationship between the concentration and percentage inhibition (Pearson's correlation analysis). [a] Mean ± SD.

Inhibition of lipid peroxidation by aqueous decoction of Nathai choori chooranam

Aqueous decoction of Nathai choori chooranam also inhibited the lipid peroxidation induced by ferrous sulfate in egg yolk homogenates. Maximum inhibition was observed with total aqueous decoction of Nathai choori chooranam with inhibition percentage 18.16 to 68.79 at 400µl/ml respectively then standard vitamin-C (Table-4). This inhibition of lipid peroxidation possibly either due to chelation of Fe or by corner of the free radicals. Iron also is playing a major role for the formation of lipid peroxidation in the body. The process of lipid peroxidation has been suggested to proceed via a free radical chain reaction, which has been associated with cell membrane damage. This membranouse damage has been suggested to contribute to various diseases, including diabetes. Incubation of egg yolk homogenates in the presence of $FeSO_4$ causes a significant increase in lipid peroxidation.

Table-1. Phytochemical screening of aqueous decoction of Nathai choori chooranam

S.No.	Phytochemical Constituents	Result indicated	Aqueous decoction of Nathai choori chooranam
1.	Alkaloids Dragendroff's reagent	Brown precipitation	+
	Mayer's reagent	Yellow precipitation	+
2.	Flavonoids Alkaline test	Yellow coloration	+
	Lead acetate	Immediate precipitation	+
3.	Polyphenols Ferrozine Test	Blue Coloration	+
4.	Terpenoids Salkowski test	Brown ring	+
5.	Tannins	Dark green blue	+
6.	Glycosides Keller-Killani test	Reddish brown ring	-
	Bronbagers Test	Pink colour in ammonia layer	-
7.	Saponins Froth Test	Foam	+
8.	Anthocynin Ammonia Test	Yellow colour in ammonia layer	+

Table-2. Inhibition of pancreatic lipase activity ofaqueous decoction of Nathai choori chooranam

Different concentration of extract	Percentage of Inhibition of pancreatic lipase activity	
	Aqueous decoction of Nathai choori chooranam	Standard Orlistat
100µl/ml	21.63±1.47	16.87±1.52
200µl/ml	34.16±1.29	29.67±2.47
300µl/ml	48.96±1.19	41.47±2.32
400µl/ml	78.92±2.47	69.93±1.19
EC_{50} value	136.48 µl/ml	141.59 µl/ml

[a] Results are expressed as percentage of Inhibition of pancreatic lipase with respect to control. Each value represents the mean+SD of three experiments.

Table 3. ABTS radical activity of aqueous decoction of Nathai choori chooranam

Different concentration of aqueous decoction of Nathai choori chooranam	[a] Percentage of radical activity with Aqueous decoction of Nathai choori chooranamon ABTS	
	Aqueous decoction of Nathai choori chooranam	Standard Vitamin-C
100µl/ml	27.89±2.13	25.63±1.87
200µl/ml	43.65±1.26**	37.45±0.46
300µl/ml	62.48±0.78	56.23±1.89**
400µl/ml	82.63±0.56	74.63±0.46
EC_{50} Value	125.12 µl /ml	138.45µl /ml

Table-4. Inhibition of lipid peroxidation induced by $FeSO_4$ using egg yolk homogenates as lipid rich media by aqueous decoction of Nathai choori chooranam

Different concentration of aqueous decoction of Nathai choori chooranam	[a]Inhibition Percentage of lipid peroxidation	
	Aqueous decoction of Nathai choori chooranam	Vitamin-C
100µl /ml	18.26±1.39	15.49±1.38
200µl /ml	29.45±0.21**	27.49±1.36
300µl /ml	42.36±1.24	38.69±1.49
400 µl /ml	68.79±1.37	60.12±1.87
EC_{50} Value	142.63µl /ml	148.63µl /ml

Table 5. Superoxide anion scavenging activity of aqueous decoction of Nathai choori chooranam

Different concentration of aqueous decoction of Nathai choori chooranam	[a] Inhibition Percentage of Superoxide anion scavenging of aqueous decoction of Nathai choori chooranam	
	Aqueous extract	Vitamin-C
100µl	25.64±0.24	23.65±1.47
200µl	36.89±2.16	31.59±2.56**
300µl	51.46±1.14	46.23±0.29
400µl	73.67±0.23**	67.69±1.25
EC_{50} Value	134.36µl /ml	143.69µl /ml

It is possible that the high level of inhibition on lipid peroxidation displayed by the ethyl acetate fraction is related to the presence of phenolic compounds, which have been correlated with antioxidant activity (Gulcin *et al*., 2002). There was a significant ($^{*}P < 0.01$) relationship between the concentration and percentage inhibition (Pearson's correlation analysis). [a]Mean ± SD.

Superoxide anion scavenging activity of aqueous decoction of Nathai choori chooranam

Superoxide radicals by photochemical decrease of nitro blue tetrazolium (NBT) in the occurrence of a riboflavin-light-NBT system, which is one of the standard methods. The aqueous decoction of Nathai choori chooranam exhibited potent

scavenging activity for superoxide radicals in a concentration dependent manner (Table-5). The aqueous decoction fraction had highest Superoxide radicals scavenging percentage 73.67±0.23 at 400µg/ml and standard Vitamin-C was least potent with 67.69±1.25 value at 400µg/ml. Removal of superoxide in a concentration dependent manner by attributed to the direct reaction of its phytomolecules with inhibition of the enzymes. All the observations in different groups showed significant ($P < 0.01$) relationship between the concentration and percentage inhibition (Pearson's correlation analysis). [a]Mean ± SD.

The present study demonstrated aqueous decoction acts as Nitric oxide scavenging due to extracts contain poly phenol compounds; free radicals are scavenged and therefore can no longer react with nitric oxide, resulting in less damage. Nitric oxide is an important chemical mediator generated by endothelial cells, macrophages, neurons and involved in the regulation of various physiological processes. At present it is increasing evidence to suggest that Nitric oxide and its derivatives produced by the activated phagocytes may have a genotoxic effect and may contribute in the multistage carcinogenesis process (Wink *et al*., 1991).

Table 6. Effect of Metal chelating activity of aqueous decoction of Nathai choori chooranam

Different concentration of aqueous decoction of Nathai choori chooranam	[a] Percentage of Metal chelating activity of aqueous decoction of Nathai choori chooranam	
	Aqueous decoction of Nathai choori chooranam	Vitamin-C
100µl	25.81±0.81	23.12±1.69
200µl	37.31±0.77	32.68±1.56
300µl	58.78±0.92**	52.36±0.23
400µl	84.08±1.51	76.56±1.36
EC_{50} Value	123.63µl /ml	136.25 µl /ml

Table 7. Nitric oxide scavenging activity of aqueous decoction of Nathai choori chooranam

Different concentration of aqueous decoction of Nathai choori chooranam	[a] Percentage of Nitric oxide scavenging aqueous decoction of Nathai choori chooranam	
	Aqueous extract	Vitamin-C
100µl	24.89±1.48	21.56±1.49
200µl	41.23±1.25**	37.89±1.63
300µl	59.63±0.46	56.21±1.49
400µl	81.04±1.67	77.41±0.63
EC_{50} Value	146.23 µl /ml	158.63 µl /ml

Effect of Metal chelating activity of aqueous decoction of Nathai choori chooranam

Table-5 shows the metal chelating effect of the aqueous decoction of Nathai choori chooranam on ferrous ions. Similarly, the ability of chelating ferrous ions also increased with the concentration ranges from 100-400µl/ml of the aqueous decoction of Nathai choori chooranam to a certain point, after that leveled off as the concentration further increased (Table-6). At a dose level of 400 µl/mL, the chelating effect of the aqueous decoction of Nathai choori chooranam could reach to 84.08% than vitamin-C.

Fe^{2+} has been shown to cause the production of oxyradicals and lipid peroxidation, minimizing Fe^{2+} concentration in Fenton reaction affords protection against oxidative damage. Chelating agents can inhibit radical generation by stabilizing transition metals, consequently reducing free radical damage. In addition, phenolic compounds have the potential to bind to metal ions due to their chemical structures, and have been shown to exhibit antioxidant activity through the chelation of metal ions (Zhao *et al*., 2008). All the observations in different extract showed significant ($P < 0.01$) relationship between the concentration and percentage inhibition (Pearson's correlation analysis). [a]Mean ± SD.

Nitric oxide scavenging assay of aqueous decoction of Nathai choori chooranam

In present study aqueous decoction of Nathai choori chooranam showed Nitric oxide scavenging activity. Good result was observed at aqueous decoction of Nathai choori chooranam with scavenging ranges 81.04±1.67 at 20 µg/ml than vitamin-C with 77.41±0.63 at 20µg/ml for vitamin C which served as positive control (Tale-7).

All the observations in different extract showed significant ($P < 0.01$) relationship between the concentration and percentage inhibition (Pearson's correlation analysis). [a]Mean ± SD.

Conclusion

Thus the results of this study indicate that the commonly used aqueous decoction of Nathai choori chooranam significantly inhibit the activity of pancreatic lipase which can be attributed to the presence of polyphenols, flavonoids tannin and saponins which is comparable to orlistat. According to data achieved from the present study, EC_{50} Value was found to be an effective antioxidant in different *in vitro* assays, including total antioxidant activity determination by ABTS radical, superoxide anion radical scavenging, Lipid peroxidation and nitric oxide scavenging when it is compared to standard antioxidant compounds used as Vitamin C. These may be used in nutraceuticals and the food industry. However, additional studies are necessary to develop a method for the fractionation and identification of polyphenols and to determine the most active antioxidant compounds in the Nathai choori chooranam.

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