Research Article

Evaluation of Physico-Chemical and Antioxidant Properties of an Indian Siddha Formulation: *Panchadeepakini choornam*

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ABSTRACT

Panchadeepakini choornam is a herbal drug prepared from five different spices (ginger, black pepper, long pepper, cardamom and cumin) and used in Siddha system of medicine to cure various gastro-intestinal problems. Even though this drug has been used based on traditional knowledge, no scientific work has been done to evaluate its physico-chemical properties and antioxidant power. Hence, in the present study, we have analyzed the physico-chemical, antioxidant and antimicrobial properties of panchadeepakini choornam using *in vitro* methods. The choornam revealed high water soluble extractive value (51.19%), bulk and tap densities of 2.49 g, total ash and insoluble ash contents of 5.24 and 0.14 %, pH value of 7.04, loss on drying value of 5.63% and particle size of 600, 425, 300 and 75 microns. Three different solvent system such as water, ethanol and methanol were employed for preparing the extract and analyzed for antioxidant capacity. The results revealed that the water extract has higher level of total phenolic concentration and maximum level of antioxidant power in terms of phosphomolybdate reducing power, ferric reducing power, radical scavenging activity against DPPH, superoxide, hydrogen peroxide and hydroxyl radicals. Hence, the results gives scientific evidence for the use of this drug in water medium, which is rich in total phenolic content and high antioxidant power in Siddha system of medicine for various gastro-intestinal problems.

Keywords: Panchadeepakini choornam, physico-chemical properties, antioxidant effect.

INTRODUCTION

Siddha medicine is considered as the oldest medical system known to mankind[1]. Evidences found in the Tamil literature indicate that the system of Siddha medicine originated in the state of Tamilnadu, Southern India[2]. The "Panchadeepakini choornam" is a Siddha medicine predominantly used for treating ailments such as flatulency, rheumatism, dizziness, indigestion, gastric trouble and ulcer[3]. The composition of this choornam includes ginger (*Zingiber officinale*), black pepper (*Piper nigrum*), long pepper (*Piper longum*), cardamom (*Elettaria cardamomum*), cumin (*Cuminum cyminum*) and cane sugar. Honey or ghee is used as vehicle for the oral administration of this medicine.

Zingiber officinale Roscoe is a popular spice belongs to the family Zingiberaceae. Traditionally ginger has been used for treating arthritis, rheumatism, sprains, constipation, indigestion, fever, infectious diseases and helminthiasis[4]. Anti-inflammatory and antioxidant properties of ginger were studied[5]. An experiment leads to the conclusion that ginger can be used for the treatment of irritable bowel syndrome[6]. Bioactive components such as gingerols, shogaol and curcumin were reported in the ginger extract[7]. *Piper nigrum* L. belongs to the family Piperaceae. Biological role of black pepper and its use as antibacterial, anti-inflammatory, anti-diarrhoeal, hepatoprotective agent were reported[8]. The antioxidant activity of this spice was studied[9]. *Piper longum* L. also belongs to the family Piperaceae. It is commomly used to treat chronic bronchitis, asthma, viral hepatitis, diarrhea, stomachache, gonorrhea, cough and tumors[10]. *P. longum* has the potential to suppress the cell proliferation and also possess antioxidant potential[11]. Piperine and piper longumine are the major alkaloids[12] which have the ability to prevent the atherosclerotic plaque formation[13].

The scientific name of cardamom is *Elettaria cardamomum* L. Cardamom produces sedative effects and is being used in the treatment of colic, diarrhea, hypertension, constipation and it is also been used as diuretic[14]. The anti-gastric ulcer effect of cardamom was reported[15]. Antioxidant activities of the cardamom extract was proved scientifically[16]. *Cuminum cyminum* L. (Cumin) belongs to the family Apiaceae. The antioxidant property of cumin was reported earlier[17]. It is used as anti-parasitic and antimicrobial agent which reduces fever and also serves to be a good pain killer. The antibacterial activity of cumin on *Klebsiella pneumonia* was studied[18]. Major components in the cumin oil are 1,8-cineole, α-terpineol, DL-limonene, nerolidol, 4-terpineol,δ-terpineol and longifolenaldehyde[19]. The cane sugar is obtained from the sugarcane (*Saccharum officinarum* L.). Sugarcane has the property of treating liver related disorders[20]. It also has the diuretic, cooling and aphrodisiac property[21] and hence used in Ayurveda to treat fatique, anaemia, ulcers etc[22].

The medicinal properties of few Siddha choornams such as anti diabetic property of Thottalvadi choornam[23], gastroprotective activity of Amukkara choornam[24] and Gokshuradi choornam[10] were analyzed previously to give scientific validation of the herbal drugs. Although the individual ingredients of Panchadeepakini choornam was

investigated for their antioxidant and gastro-intestinal protective effects, the physic-chemical standards, antioxidant and antimicrobial effects of this choornam is not yet analyzed. So, in the present study, the physicochemical and antioxidant effects of the different extracts of Panchadeepakini choornam is investigated.

MATERIALS AND METHODS

Sample preparation

The Panchadeepakini choornam was prepared according to the method described by the Siddha Formulary of India[3]. All the ingredients (ginger, black pepper, long pepper, cardamom and cumin) were roasted gently at a temperature of 45-50°C and cooled to the room temperature (37°C). Each of the ingredients is powdered separately in a commercial mixer (Make: Daspan, India). Finally the finely powdered ingredient (100 grams each) was mixed with 500 grams of cane sugar and the prepared choornam is used for further analysis.

Physico-chemical analysis

Particle size analysis

The particle size was analysed by the method described[25]. Various sieve size of 600, 425, 300 and 75 microns were used and different size of the particles present in the choornam was analysed.

Density

The bulk density and the tapped density of the choornam were investigated by following the method of TIP[26]. Dry powder form of the choornam (2.5 g) was measured and taken in a graduated cylinder and the tap density and the bulk density were recorded.

pH value

The pH of the choornam was measured using the pH meter at 24.4°C for 1% (Weight of sample per Volume) solution.

Ash content

The determination of the total ash content of Panchadeepakini choornam was done[27]. Chooram (1.0896 g) is added to a pre-weighed silica crucible and heated in the muffle furnace at 400 C for about 3 h. Then the crucible was safely placed in the dessicator and allowed to cool to room temperature and the weight is finally measured. The percentage weight of the ash is calculated using the formula, Weight of the ash / Weight of the drug taken x 100. The percentage of acid insoluble ash is calculated using the formula, Weight of the residue Weight of the choornam x 100, where the weight of the residue is the net weight of ash.

Loss on drying

To a pre-weighed dish, 1.0605 g of choornam was added and placed in the hot plate at a temperature of 105°C. The percentage weight of the choornam after loss on drying is calculated by using the formula, Weight of the dish before LOD - Weight of the dish after LOD / Weight of the sample taken x 100.

Extractive value

The alcohol and water soluble extractives of the choornam were analyzed[27]. Dry powder of choornam (1.0034 g) was taken in two beakers separately and 50 ml of alcohol in the first beaker and water in the second one and shaken well manually. The beakers were kept aside for 24 hours and thereafter 10 ml of the solution was taken and kept in hot air oven at 105°C. Finally the percentage weight of the extract is calculated using the formula, Weight of residue / Weight of the choornam * 100.

Total phenolic content

For preparing extract, 5 g of dry powdered choornam was taken with 50 ml of ethanol, methanol and water separately in individual conical flasks. The mixer was kept under magnetic stirring for 1 h at room temperature (37°C). Then the contents were filtered through a filter paper placed on the funnel and the volume of the extract was noted. The volume of methanolic, ethanolic and water extract of choornam was 41.5, 38.0 and 42.5 ml, respectively. The above obtained extracts were used to carry out the following tests. The total phenolic content was analyzed using Folin-Ciocalteu reagent method with some modifications[28]. The sample (50 µl opportunely diluted) is added to 250 µl of Folin-Ciocalteau reagent in a test tube and vortexed. Then, 4.7 ml of 2.2% sodium carbonate solution are added and the mixture is vortexed again. A blank is prepared with 50 µl of the sample solvent instead of the sample. The tubes are incubated at 40°C for 30 min in the dark. The absorbance is read at 750 nm against the blank using Spectrophotometer (Perkin-Elmer, Model). A calibration curve was prepared with standard ferulic acid (200 – 1600 mg/L, R^2 = 0.9978) and used to express the results as ferulic acid equivalents (FAE). The total phenolic content of the sample was then calculated and expressed on dry weight and fresh weight basis.

Antioxidant activity

Phosphomolybdate assay

The antioxidant activity of extracts was evaluated[29] by taking an aliquot of 100 µl of extract was combined with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) in a screw-capped vial. The vials were closed and incubated in a water bath at 95 C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The results expressed as ascorbic acid equivalent antioxidant activity.

Ferric reducing power

The reducing power of extract was determined[30] by taking the sample (2.5 ml) in phosphate buffer (2.5 ml,

0.2 M, pH 6.6) were added to potassium ferricyanide (2.5 ml, 1.0%) and the mixture was incubated at 50 C for 20 min. Trichloroacetic acid (2.5 ml, 10%) was added, and the mixture was centrifuged at 650 x g for 10 min. The supernatant (5.0 ml) was mixed with ferric chloride (5.0 ml, 0.1%), and then the absorbance was read spectrophotometrically at 700 nm. Based on the absorbency value, the ferric reducing power of extract was expressed.

DPPH radical scavenging activity

The DPPH radical scavenging activity was analyzed[31] by taking the extract (100 µl) and added to 3.9 ml of DPPH solution (0.025 g/L) and the reactants were incubated at 25 C for 30 min. Different concentrations of ferulic acid was used as a positive control and ethanol was used instead of extract in blank. The decrease in absorbance was measured at 515 nm with a spectrophotometer. The radical scavenging activity of tested samples was calculated and expressed on percentage basis.

Superoxide radical scavenging activity

The capacity of extracts to scavenge the superoxide anion radical was measured[32]. The reaction mixture was prepared using 3 x 10^{-6} M riboflavin, 1 x 10^{-2} M methionine, 1 x 10^{-4} M nitroblue tetrozolium chloride and 0.1 mM EDTA in phosphate buffered saline (pH 7.4). For the analysis, 3.0 ml of the reaction mixture was taken with 100 µl of extract in closed tubes and illuminated for 40 min under fluorescent lamp (18 W). The absorbance was then read at 560 nm against the un-illuminated reaction mixture. Results are expressed as superoxide radical scavenging activity on percentage basis.

Hydrogen peroxide scavenging activity

The effect of extracts on hydrogen peroxide was analyzed[33] by taking the extract (100 microliter) and mixed with 5 ml of 45 mM hydrogen peroxide solution in 0.1 M phosphate buffer (pH 7.4). The reaction mixture was vortexed and incubated for 30 min at room temperature and then the absorbency was measured at 230 nm. The extract with phosphate buffer is used as a blank and the level of hydrogen peroxide remaining in the solution was calculated using a calibration curve. The hydrogen peroxide inhibition effect of extract was calculated and expressed on percentage basis.

Hydroxyl radical scavenging activity

The hydroxyl radical quenching activity of extracts was evaluated[34]. The reaction mixture consists of 10 mM phospate buffer (pH 7.4), 2.8 mM Deoxyribose, 2.8 mM H2O2, 0.025 mM FeCl3, 0.1 mM EDTA and 0.1 mM ascorbic acid in a total volume of 3 ml. With the reaction mixture, 100 microliter of extract was added and incubated at 37 C for 15 min. Then the reaction was terminated by the addition of 1 ml of 2.5% ice-cold TCA and 1% TBA. The reactants were mixed well and heated at 90 C for 15 min in a water bath and cooled to room temperature. The chromogen was extracted with 1-butanol and absorbency was measured at 530 nm. Based on absorbency value, the hydroxyl radical scavenging activity of extracts was calculated and expressed on percentage basis.

RESULTS AND DISCUSSION

Physico-chemical properties

Table 1: Physico-chemical properties of Panchadeepakini choornam.

S. No.	Parameter	Physico-chemical properties
1	Evaporation (Residue weight)	0.092 g
2	Alcohol soluble extractive value (% weight)	10.61 %
3	Water soluble extractive value (% weight)	51.19 %
4	Bulk density (weight)	2.49 g
4	Bulk density (volume)	4.5 ml
5	Tap density (weight)	2.49 g
5	Tap density (volume)	3.9 ml
6	Total ash content (% weight)	5.24 %
7	Acid insoluble ash (% weight)	0.14 %
8	pH at 24.4°C	7.04
9	Loss on drying (% weight)	5.63 %
10	Particle size 600 microns 425 microns 300 microns 75 microns	 2.32 g 2.16 g 1.09 g 4.38 g

The physico-chemical properties of panchadeepakini choornam were shown in the Table 1. The panchadeepakini choornam has higher level of water soluble extractives (51.19%) than alcohol soluble extractives (10.61%), which explains the traditional use of this drug in aqueous medium. The choornam revealed similar level of bulk and tap densities of 2.49 g on weight basis, but different bulk and tap density of 4.5 ml and 3.9 ml, respectively by volume basis. The presently investigated choornam revealed 5.24 and 0.14 % of total ash and insoluble ash contents, respectively. The pH value of 1% solution of the choornam was found to be neutral (7.04), so that its direct use is not harmful to human beings. The choornam exhibited loss on drying value of 5.63 % on percentage basis. The particle size analysis reveals that the panchadeepakini choornam is constituted with particles of 2.32, 2.16, 1.09 and 4.38 g of 600, 425, 300 and 75 microns and about 50% of the particles have low diameter of 75 microns.

Total phenolic content

The total phenolic concentration of different solvent extracts of panchadeepakini choornam was given in the Figure 1. The total phenolic compounds were quantified by Folin-Ciocaltue reagent method. This assay is based on the reduction of Folin's reagent by the phenolic compounds. Under alkaline pH, phenols dissociate into

phenolate anion and proton, hence phenolate anion can reduce the Mo (VI), which is a major component of Folin's reagent, by single electron transfer. Due to this reduction, a blue coloured complex $(PMoW_{11}O_{40})^{4-}$ is formed with absorption max at 750 nm. Among the studied extracts, the water extract of panchadeepakini choornam exhibited higher level of total phenolic concentration (452.86 mg FAE/L), which is followed by ethanolic extract (320 mg FAE/L) and methanolic extract (245.71 mg FAE/L).

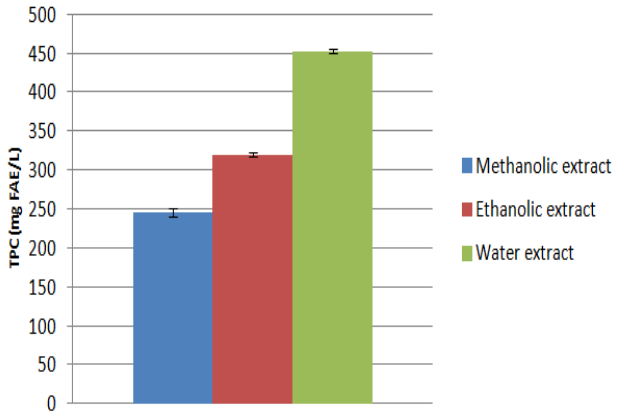

Figure 1: Total phenolic concentration of different solvent extracts of Panchadeepakini choornam

Phosphomolybdate reducing power

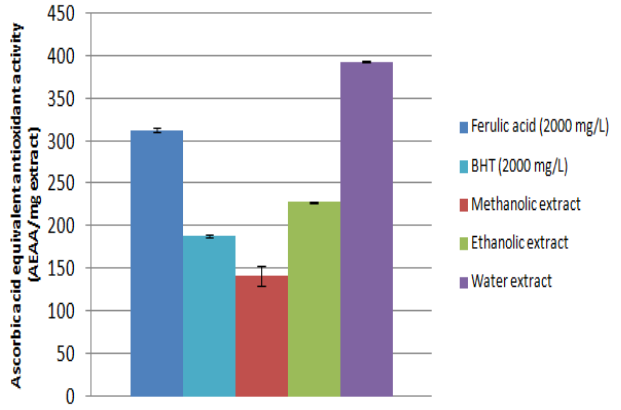

Figure 2: Phosphomolybdate reducing power of Panchadeepakini choornam

The phosphomolybdate reducing power of different solvent extracts of panchadeepakini choornam was shown in Figure 2. Among the different extracts, the water extract revealed higher level of reducing power (392.75 AEAA.mg extract) than that of ethanolic extract (228 AEAA.mg extract), methanolic extract (141.5 AEAA.mg extract), the reference compounds ferulic acid (312.5 AEAA.mg extract) and BHT (188 AEAA.mg extract). When the molybdenum (VI) is reduced to Mo (V) by an antioxidant, it forms a green colored complex at acidic pH in the presence of phosphorous with the absorption maxima at 695 nm. This assay evaluate the reducing or electron donating power of the antioxidant to Molybdenum and the intensity of PMo(V) complex is proportional to antioxidant power of the extract. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant.

Ferric reducing power

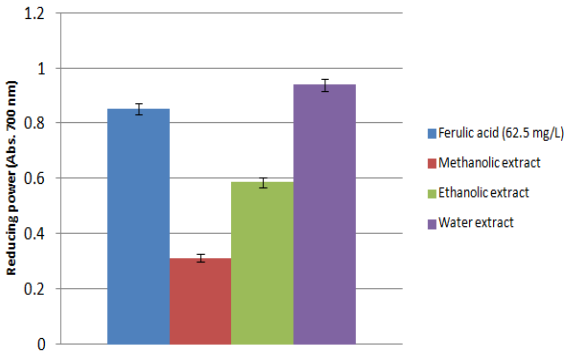

Figure 3: Ferric reducing power of Panchadeepakini choornam

In ferric reducing assay, Fe (III) is reduced to Fe (II) by the antioxidant compound through electron transfer. The reduced Fe (II) forms the Pearl's blue complex, which can be measured at 700 nm. Figure 3 describes the ferric reducing power of different solvent extracts of panchadeepakini choornam. The water extract of panchadeepakini choornam demonstrated higher level of reducing power (0.939 Abs units), which is followed by the reference compound ferulic acid (0.85 Abs units), ethanolic extract (0.58 Abs units) and water extract (0.31 Abs units).

DPPH radical scavenging activity

The DPPH radical scavenging activity of different solvent extracts of panchadeepakini choornam was illustrated in the Figure 4.

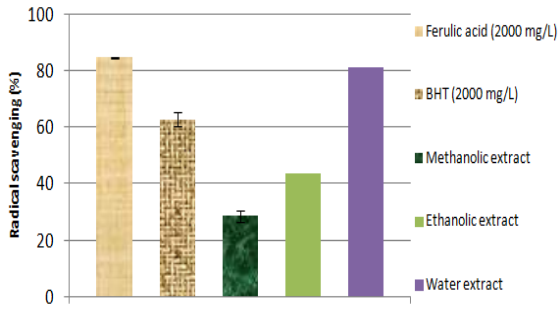

Figure 4: DPPH radical scavenging activity of Panchadeepakini choornam

The evaluation of the antioxidant power by DPPH radical scavenging activity has been widely in use for different plant extracts. DPPH (2,2-Diphenyl-1-picrylhydrazyl) is a stable radical, methanolic solution of which has dark purple color with maximum absorption at 515 nm**.** Antioxidants can reduce DPPH through hydrogen transfer into its non-radical form (DPPH-H) and hence the absorption disappears at 515 nm. The decrease in absorbency at 515 nm may be due to the reaction between phytochemicals and DPPH, which indicates the

antioxidant power. The water extract of panchadeepakini choornam revealed higher level of radical scavenging power (81.04%) than ethanolic exctract (43.33%), methanolic extract (28.45%), synthetic antioxidant BHT (62.69%), but lower than reference compound ferulic acid (84.67%).

Superoxide radical scavenging activity

The superoxide radical scavenging activity of samples was investigated by generating superoxide through photo-induced reduction of riboflavin, which can generate superoxide radical in the presence of methionine. The generated superoxide radical reduce the NBT into purple colour formazan, which was measured at 560 nm. In presence of antioxidant, the generated superoxide radicals were scavenged and hence, formation of purple colour formazan is minimum or nil. The superoxide radical scavenging activity of solvent extracts of panchadeepakini choornam was shown in Figure 5. Among the different solvent extracts analyzed in the present study, the water extract recorded maximum level of superoxide radical scavenging activity (85% at 1000 g/L) than ethanolic extract 50% at 1000 g/L) and methanolic extract (20% at 1000 g/L). However, the superoxide radical scavenging activity of water extract was lower than that of reference compound ferulic acid (98% at 1000 g/L).

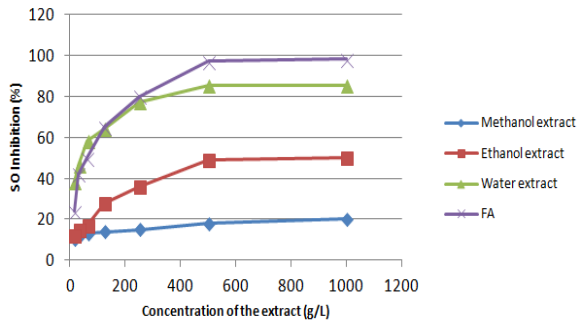

Figure 5: Superoxide radical scavenging activity of Panchadeepakini choornam

Hydrogen peroxide inhibition

Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells. Thus, removing hydrogen peroxide is very important for protection of cellular system. The hydrogen peroxide can decompose into water by accepting two electrons and protons. The level of hydrogen peroxide in buffer solution can be detected spectrometrically at 230 nm. If antioxidants (electron donors) are added to the reaction mixer, they can accelerate the conversion of hydrogen peroxide into water. Figure 6 reveals the hydrogen peroxide inhibition activity of different solvent extracts of panchadeepakini choornam. The water extract registered maximal level of hydrogen peroxide inhibition activity (76% at 1000 g/L) than that of ethanolic extract (40% at 1000 g/L), methanolic extract (25% at 1000 g/L) and the reference compound, ferulic acid (72% at 1000 g/L).

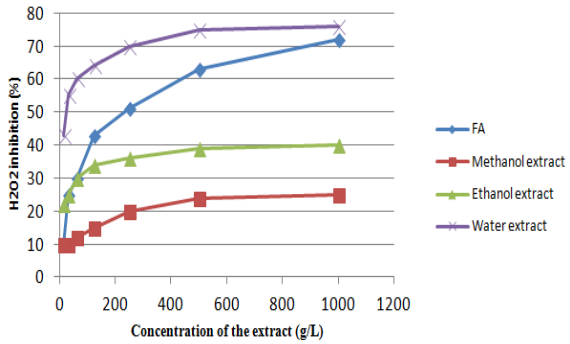

Figure 6: Hydrogen peroxide inhibition activity of Panchadeepakini choornam

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of different solvent extracts of panchadeepakini choornam was presented in the Figure 7.

Among the different extracts investigated in the present study, the water extract exhibited maximum level of hydroxyl radical inhibition activity (81% at 1000 g/L), which is followed by the reference compound, ferulic acid (64% at 1000 g/L), ethanolic extract (30% at 1000 g/L) and methanolic extract (12% at 1000 g/L).

Hydroxyl radicals are produced by the Fenton reaction between Fe(II)-EDTA and hydrogen peroxide. The hydroxyl radicals (OH.) degrade Deoxyribose and produce MDA, which can be measured by TBARS reaction.

The TBA can react with MDA in acidic medium to form pink colour chromogen, which could be extracted with 1-butanol and read at 530 nm. OH radicals may attack various biomolecules including proteins, lipids, and DNA and cause oxidative damage to the cellular components and hence it is considered to be biologically dangerous free radical.

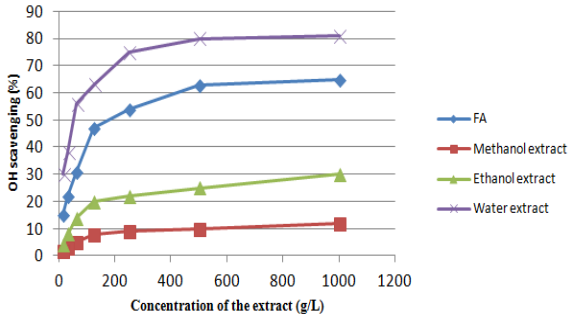

Figure 7: Hydroxyl radical scavenging activity of Panchadeepakini choornam

CONCLUSION

From the findings of the present study, the physico-chemical and antioxidant properties of panchadeepakini choornam was revealed scientifically. The Siddha formulation, panchadeepakini choornam was studied for the physico-chemical properties for the first time and the standards such as extractive value, density, total and acid insoluble ash contents, pH, loss on drying and particle size

were determined and reported. Further, the water extract of panchadeepakini choornam revealed higher level of total phenolic concentration and antioxidant property based on various *in vitro* assays. This finding supports the use of this choornam in water medium in Siddha system of medicine to cure various stomach related problems. The high antioxidant property exhibited by the water extract of this choornam provides scientific support for employing this herbal drug for therapeutic use in Indian system of medicine.

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