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Antioxidant activity of siddha polyherbomineral drug Gandhaga Rasayanam

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Abstract

Gandhaga Rasayanam a polyherbo mineral drug mainly useful in treating vatha diseases. Antioxidant activity of Gandhaga Rasayanam is being tested scientifically by using the samples of prepared Gandhaga Rasayanam. The qualitative and quantitative analysis of antioxidant activity of Gandhaga Rasayanam extract was determined. Phenols and flavonoids which are being considered as the antioxidant compounds are also evaluated.

Keywords: Siddha Medicine, Gandhaga Rasayanam, Phytochemicals, Antioxidant activity.

Introduction

Siddha system of medicine is an ancient system of medicine which is developed and enriched by paramount power called siddhars. Siddha mainly deals with ashtama siddhiie 8 super natural powers. Siddhars have an enormous amount of knowledge regarding chemicals, metals, minerals and plants, which were successfully used by them for various purposes in various conditions/ situations.

Gandhaga Rasayanam a polyherbo mineral drug is one among them. It is mainly useful in treating vatha disorders like Rheumatoid arthritis. It is also useful for treating Gunmam, Pandu, Sohail, Kshayam, Meham, Agnimandham, Vatham (80), Pitham (40), Soolai (18), Kiruchanam (6). Rheumatoid Arthritis is an auto immune disorder commonly occurs in small joints [inter phalangeal joints] initially, then gradually develops and affects larger joints [hip / knee joints]. About 180 million people [approximately] in India is being suffering from Rheumatoid arthritis. Anti oxidant compounds are capable of protecting against oxidative damage by decreasing the number of free radicals which cause chronic diseases.

Highly reactive oxygen species or free radicals are being capable of inducing oxidative damage to human body. Anti oxidants are the compounds which terminate the attack of reactive species and reduce the risk of diseases. The antioxidant activity of Gandhaga Rasayanam extract was determined by following the method as described by George et.al (1996); Selvaraj et.al (2013).

"*Ghanthagam*" or Sulphur is one among the *Pasanam*. In the present paper Process Standardization Studies on Ghanthagam Rasayanam is taken up which is prescribed in various diseases such as gastric ulcers, chronic wounds, other skin problems such as leprosy and in Leucoderma, tumors for which modern medicine has no cure. (Rajalakshmi et al., 2012).

Kandhaga Rasayanam is a Siddha drug chosen from the classical Siddha text Siddha Vaidhya Thirattu (Rajalakshmi, 2010). It is indicated for skin diseases, urinary tract infections, venereal diseases, arthritis etc. (Kuppuswamy et al., 1998). The present study was

intimated analysis of Anti-oxidant activity of siddha polyherbomineral drug Gandhaga Rasayanam.

The above prepared medicine is preserved in a pot and kept in Nerpudam for 10days.

Materials and Methods

Medicine preparation:

Ingredients:

Piper nigrum,
Zingiber officinalis,
Piper longum;
Syzygium aromaticum;
Elettaria Cardamomum;
Cinamomum verum;
Cinnamomum tamala ;
Myristica fragans;
Abies spectabilis;
Mesua nagassarium;
Plectranthus vettiveroides;
Trachyspermum roxburghianum;
Cuminum cyminum;
Hyoscyamus niger;
Curcuma zedoaria;
Alpinia galanga;
Plumbago indica;
Scindapsus officinalis;
Nigella sativa;
Maranta arundinacea;
Santalum album;
Anacardium occidentale;
Illicium verum
Glycyrrhiza glabra;
Cyperus rotundus;
Nardostachys grandiflora;
Coriandrum sativum;
Phoenix dactylifera;
Three myrobalans;
Vetivera zizanoides;
Crocus sativus;
Curculigo orchioides;
Withania somnifera;
Smilax chinensis;
Mucuna pruriens;
Hydrophylla auriculata;
Tribulus terrestris;
Asparagasus racemosus.

Sugar; Ghee; Honey; Purified Sulphur.

Preparation:

The raw drugs are collected and dried in sunshade it is then pulverised to make a fine powder (Chooranam). Sugar syrup is made with thaneervittankizhanguchaaru (*Asparagasus racemosus*).

Sulphur purified with cow's ghee and chooranam is added to sugar syrup and stirred well. The mixture is allowed to cool and then honey is added.

Dose:

5gm twice a day with hot water

Duration:

40 days

Preparation of the plant extract:

Preparation of the extracts was assessed by following method as described by Janarthanam *et al.*, 2013. 0.5 gram of Gandhaga Rasayanam dried powder of product materials were extracted with 20 mL aqueous for 1 min using an Ultra Turax mixer (13,000 rpm) and soaked overnight at room temperature. The sample was then filtered through Whatman No. 1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota-vator at 40 °C to a constant weight and then dissolved in respective solvents. The dissolving rate of the crude extracts was approximately 100 %. The solution was stored at 18 °C until use.

Antioxidant Activity of Gandhaga Rasayanam extracts

Qualitative analysis of antioxidant activity of Gandhaga Rasayanam extract

The antioxidant activity of Gandhaga Rasayanam extract was determined by following the method as described by George *et al.*, (1996); Selvaraj *et al.*, (2013). 50µl of Gandhaga Rasayanam extracts were taken in the microtiter plate. 100µl of 0.1% methanolic DPPH was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively. The antioxidant positive samples were subjected for further quantitative analysis.

Quantitative analysis of free radical scavenging activity of Gandhaga Rasayanam extract

The antioxidant activities were determined using DPPH (Sigma-Aldrich) as a free radical. 100µl of Gandhaga Rasayanam extracts were mixed with 2.7ml of methanol and then 200µl of 0.1 % methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control (Lee *et al.*, 2005). Subsequently, at every 5 min interval, the absorption maxima of the solutions were measured using a UV double beam spectra scan (Chemito, India) at 517nm. The antioxidant activity of the sample was compared with known synthetic standard of 0.16% Butylated

Hydroxy Toluene (BHT). The experiment was carried out in triplicates. Free radical scavenging activity was calculated by the following formula:

$$\% \text{ DPPH radical-scavenging} = \frac{[(\text{Absorbance of control} - \text{Absorbance of test Sample}) / (\text{Absorbance of control})] \times 100}{100}$$

Determination of Total Phenolic Contents in Gandhaga Rasayanam

Total phenolic content in the aqueous Gandhaga Rasayanam extracts was determined by the Folin Ciocalteu colorimetric method (Slinkard and Singleton, 1984). For the analysis, 0.5 ml aliquot of sample was added to 0.5 ml of Folin- Ciocalteu reagent (0.5N) and the contents of the flask were mixed thoroughly. Later 2.5 ml of sodium carbonate (2%) was added, and the mixture was allowed to stand for 30 minutes after mixing. The absorbance was measured at 760 nm in a UV-Visible Spectrophotometer. The total phenolic contents were expressed as mg gallic acid equivalents (GAE)/g extract.

Estimation of Total Flavonoid Content in Gandhaga Rasayanam

Total flavonoids content in the aqueous Gandhaga Rasayanam extracts was determined by the aluminium chloride colorimetric method (Mervat *et al.*, 2009). 0.5 ml of Gandhaga Rasayanam extracts at a concentration of 1mg/ ml were taken and the volume was made up to 3ml. Then 0.1ml AlCl₃ (10%), 0.1ml of potassium acetate and 2.8 ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance was recorded at 415 nm after 30 minutes of incubation. A standard calibration plot was generated at 415 nm using known concentrations of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent (QE) /g of sample.

Results and Discussion

Table 1 Composition of Gandhaga rasayanam extract

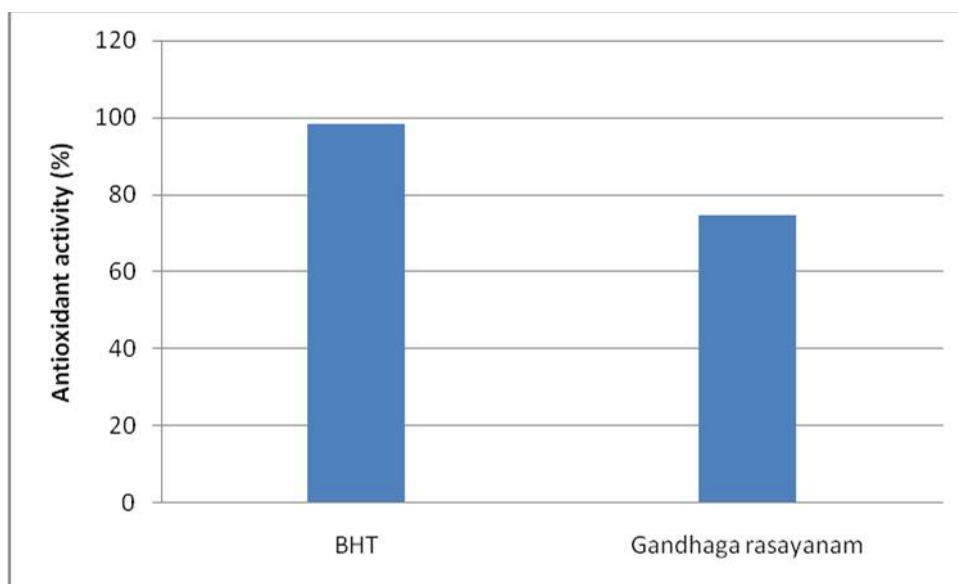
S.No	Tamil Name	Botanical Name	quantity
1	Thirikaduku	<i>Zingiber officinalae</i> <i>Piper nigrum</i> <i>Piper longum</i>	16.8gm each
2	Kirambu	<i>Syzygium aromaticum</i>	16.8gm
3	Elam	<i>Elattaria cardamomum</i>	16.8gm
4	Elavanghapattai	<i>Cinnamomum verum</i>	16.8gm
5	Elavanghapatri	<i>Cinnamomum tamale</i>	
6	Sathikkai	<i>Myristica fragrans</i> Henlt	16.8gm
7	Omam	<i>Carium copticum</i>	16.8gm
8	Jeerakam	<i>Cuminum cyrinum</i> Linn	16.8gm
9	Ponaikkali	<i>Mucuna pruriens</i> Linn Dc.	16.8gm
10	Neermulli	<i>Hygrophylla auriculata</i>	16.8gm
11	Nerunjil	<i>Tribulus terrestris</i> Linn	16.8gm
12	Ashwagandhi kizhanghu	<i>Withania somnifera</i> Linn	16.8gm
13	Kumkuma poo	<i>Crocus sativus</i> Linn	16.8gm
14	Sathipathri	<i>Myristica fragrans</i> Henlt	16.8gm
15	Thiripala	<i>Terminalia chebula</i> <i>Terminalia bellarica</i> <i>Embelica officinalis</i>	16.8gm each
16	Perichampalam	<i>Phoenix sylvestris</i>	16.8gm
17	Kothamali	<i>Coriandrum sativum</i>	16.8gm
18	Sadamanjil	<i>Nardostachys grandiflora</i> Dc	16.8gm
19	Athimadhuram	<i>Glycyrrhiza glabra</i> Linn	16.8gm
20	Takkol	<i>Illicium verum</i>	16.8gm
21	Sandanum	<i>Santalum album</i> Linn	16.8gm
22	Munthirikkai	<i>Anacardium occidentale</i> Linn	16.8gm
23	Karunjeerakam	<i>Nigella sativa</i> Linn	16.8gm
24	Paranghipattai	<i>Smilax chinnensis</i> Linn	700gm
25	Anaithippili	<i>Scindapsus officinalis</i> Schott	16.8gm
26	Thippili moolam	<i>Piper longum</i>	16.8gm
27	Citramoolam	<i>Plumbago indica</i> Linn	16.8gm

28	Kurosaani omam	<i>Hyoscyamus niger</i> Linn	16.8gm
29	Vilamichhu veer	<i>Plectranthus vettiveroides</i>	16.8gm
30	Sirunagha poo	<i>Mesua nagassarium</i> Kosterm	16.8gm
31	Talisa patri	<i>Abies spectabilis</i> Mirb	16.8gm
32	Poolangkizhangu	<i>Curcuma zedoaria</i> Mirb	16.8gm
33	Vettiver	<i>Vetiveria zizanoioides</i> Rosc	16.8gm
34	Thannervittankizhanghuchaaru	<i>Asparagus recemosa</i> Wild	7.81litre
35	Koovai neer	<i>Maranta arundinaceae</i> Linn	16.8gm
36	Nilapanai kilanzhu	<i>Curculigo orchioides</i>	16.8gm
37	Muthakkasu	<i>Cyperus rotundus</i> Linn	16.8gm
38	Sitrathai	<i>Alpinia galangal</i> Linn	16.8gm
39	Sarkarai	Sugar	2450g
40	Gandhagam	Sulphur	140g
41	Then	Honey	1.31 litre

Table 2 Qualitative antioxidant activity of Gandhaga Rasayanam extract

S.No	Extractions Control	Gandhaga Rasayanam
	BHT (standard)	++
S1	Aqueous	+

Figure 1 Quantitative analysis of free radical scavenging activity of Gandhaga Rasayanam extract



BHT – standard – 98.4%

Gr – sample – 74.8 %

Table 3 . Phytochemical study of Gandhaga Rasayanam

Quantification Phytochemical	Gandhaga Rasayanam
Total phenolic content (mg GAE/g)	0.182
Total flavonoid content (mg GAE /g)	0.357

Flavonoids are phytoconstituents which contains a large group of polyphenolic compounds having a

benzo-Y- prone structure. Lipids are protected by flavonoids against oxidative damage. Flavonoids have

an ability to reduce free radical formation and to cruise free radicals. The polyphenolic constituents obtained from plants are more effective antioxidants *in vitro* than vitamin-E & C. functional hydroxyl groups present in flavonoids negotiate their anti-oxidant effects by cruising free radicals or by cheating metal ions.

Conclusion

Gandhaga Rasayanam has proved its antioxidant activity. The antioxidant compounds such as phenols and flavonoids were found in Gandhaga Rasayanam. Hence, Gandhaga Rasayanam stops further degeneration and management of Rheumatoid arthritis.

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