



Research Article

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A COMPARATIVE STUDY ON THE ANTIOXIDANT ACTIVITY OF TWO CONVENTIONAL AYURVEDIC FORMULATIONS CONTAINING *TERMINALIA ARJUNA*: PARTHA ARISHTA AND ARJUNA GHRITA

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ABSTRACT

In the present study, two Ayurvedic formulations were prepared and their antioxidant activity was evaluated. The materials required for the synthesis were collected from Thiruvananthapuram District, Kerala. Two formulations Arjuna arishta and Arjuna ghrta were prepared as per traditional Ayurvedic methods. Physico-chemical properties of both formulations were analyzed. Antioxidant activity of the formulations was evaluated by nitric oxide scavenging activity and DPPH (2, 2-diphenyl -1-picrylhydrazyl) assay method. Results of organoleptic and Physico-chemical evaluation of formulations showed that they are good products and palatable to use. In case of antioxidant activity, both formulations showed activity in both methods of evaluations. But comparing with arishta, the ghrta formulation showed a better activity in both methods of evaluation. These results are useful for the further investigation in the future.

Key words: Partha arishta, Arjuna ghrta, *In vitro* antioxidant activity, Nitric oxide scavenging activity, DPPH assay

INTRODUCTION

Increasing awareness about the adverse effects of modern medicines leads to a great interest in Ayurveda at international level as well as within India. Generally, Ayurveda remedies are less cost and safer one. This traditional healing system comprises of various types of formulations including arishtas (a fermented form)¹ and ghrtas (medicated ghee)².

Terminalia arjuna, commonly known as arjuna belongs to the family of Combretaceae³ is a source plant of Ayurvedic classical drug. According to Charaka Samhita, arjuna is useful in the management of Urdara (urticaria). The Vrindamadhava (9th AD), a medieval compendium recommended it for the treatment of Hridroga (heart disease)⁴. Recently, investigations about the mechanism of cardio protective effect of arjuna shown a dose dependent regulation of blood pressure and heart rate. There was also a slight increase in the HDL to total cholesterol ratio and an overall improvement in the cardiovascular profile⁵. It possesses antibacterial activity and also helps in promoting proper gastro intestinal function⁶.

In the present study, we take a decision to compare the antioxidant activity of Partha arishta (Arjuna arishta) and Arjuna ghrta, an attempt to provide a direction for further research.

MATERIALS AND METHODS**Materials collection and identification**

Terminalia arjuna (Combretaceae) stem bark, Dried fruits of *Vitis vinifera* (Vitaceae), Roots of *Glycyrrhiza glabra* (Leguminosae), Flowers of *Madhuka indica* (Sapotaceae) and *Woodfordia forticosa* (Lythraceae) were collected from the Thiruvananthapuram District, Kerala. Taxonomic identification of collected materials was done in the Department of Botany, Christian College, Kattakada, Thiruvananthapuram District, Kerala. Jaggery was procured from the local market.

Preparation of Partha arishta (Arjuna arishta)

The collected drug materials were cleaned, washed, dried and used for the preparation. Composition of Partha arishta was shown in table 1.

Table 1: Composition of Partha arishta

No.	Ingredients	Quantity
1	<i>Terminalia arjuna</i> stem bark	4.80 kg
2	<i>Vitis vinifera</i> fruits	2.40 Kg
3	<i>Madhuka indica</i> Flowers	0.96 Kg
4	<i>Woodfordia forticosa</i> Flowers	0.96 Kg
5	Jaggery	4.80 kg
6	Water	49.152 L

Preparation of decoction

For the preparation of arishta, initially decoction of *Terminalia arjuna* bark was prepared. For that, the pulverized *Terminalia arjuna* bark was passed through the sieve no.44 and mixed with suitable quantity of water and kept overnight. Then the mixture was subjected to mild heating till the quantity gets reduced to one fourth of the initial volume. Now the decoction obtained was filtered through muslin cloth and the filtrate collected was used for the further process.

Fermentation

Suitable quantity of jaggery was dissolved in the decoction by stirring and filtered by using muslin cloth. Filtrate was collected in a clean porcelain jar and the remaining ingredients were added to it and mixed well. Then the mouth of the container was sealed with mud smeared cloth and kept in clean and dry room for one month for fermentation. After fermentation the fermented liquid (Arishta) is decanted and filled in the air tight container and used for the further evaluation.

Preparation of Arjuna ghrita

Bark of arjuna (1 kg) is cooked in water (Jala 8/16 kg) till the quantity was reduced to one fourth of the initial volume (padavasesa). Then it is filtered and re cooked in Cow's ghee (ghrita 1 kg) by adding paste (kalka) of Liquorice (root paste 250 gm). Then the whole content was heated for three days consecutively for the evaporation of water content and leave behind only the ghrita portion which was then filtered by a thin cotton cloth and finally the Arjuna ghrita obtained was preserved for further evaluation.

Physico-chemical evaluation of formulations

General physico-chemical parameters such as organoleptic characters, Test for the absence of methanol, Determination of pH, Determination of alcohol content and Estimation of total solids in the Arjuna arishta were determined as per the procedures prescribed in the Ayurvedic Pharmacopoeia of India. In case of Arjuna ghrita, organoleptic characters, Test for rancidity and specific gravity, acid value and saponification value were determined.

Evaluation of *in vitro* antioxidant activity**Nitric oxide scavenging activity**

Nitric oxide scavenging activity was measured spectrophotometrically. Sodium nitroprusside (5mM) in phosphate buffered saline (pH 7.4) was mixed with the different volumes of formulation subjected to evaluation (12.5-200µl) and incubated at 25°C for 2.5 h. Normal control without test agent, but an equal amount of distilled water and gallic acid as a standard control were prepared and incubated for comparative evaluation. After incubation, 1.5ml of the incubated solution was taken and diluted with 1.5ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% N-1-naphthyl ethylene diamine dihydrochloride). Absorbance of the chromophore formed during diazotization of the nitrate with sulphanilamide and subsequent coupling with N-1 naphthyl ethylene diamine dihydrochloride was measured at 546 nm and the percentage scavenging activity was analyzed.

$$\% \text{ inhibition} = \frac{\text{Control} - \text{test}}{\text{control}} \times 100$$

DPPH (2, 2-diphenyl -1-picrylhydrazyl) assay

Different volumes (1.25-20 µl) of test agents were made up to 40 µl with DMSO and 2.96 ml DPPH (0.1 mM) solution was added to it. Then the reaction mixture was incubated in dark condition at room temperature for 20 minutes. After that, the absorbance of the mixture was read at 517 nm and the percentage scavenging activity was analyzed. 3ml of DPPH was used as normal control and ascorbic acid was used as standard control.

$$\% \text{ inhibition} = \frac{\text{Control} - \text{test}}{\text{control}} \times 100$$

RESULTS

In the present study, the Ayurvedic formulations Arjuna arishta (Partha arishta) and Arjuna ghrita were prepared by traditional Ayurvedic methods. The prepared arishta and ghrita were evaluated for their organoleptic characteristics (Table 2). In case of physico-chemical evaluation of Arjuna arishta, the results showed that the methanol content was absent in the formulation. The pH analysis showed that it was 4 and its alcohol content was 8% w/w. It showed 7% w/w total solid content and 0.9970 specific gravity (Table 3).

The physico-chemical evaluation of Arjuna ghrita revealed that there was no rancidity in the formulation. The acid value determination showed that it was 1.45. In case of saponification value, it was 182. The specific gravity determination showed that it was 0.827 (Table 4).

Regarding with the evaluation of *in vitro* antioxidant activity of formulations, the results of nitric oxide scavenging activity are shown in table 5. It showed that the Arjuna arishta exhibited a maximum scavenging activity of 48.73% and the Arjuna ghrita formulation showed a maximum scavenging activity of 50.84% at the concentration of 200 µl. In the same concentration, the standard control gallic acid showed 91.59% activity (Table 6).

In case of DPPH assay, the results showed that, the Arjuna arishta showed a maximum DPPH scavenging activity of 32.33% and Arjuna ghrita exhibited a maximum DPPH scavenging activity of 71.65% at the concentration of 20 µl whereas the standard control ascorbic acid showed a maximum activity of 93.24% at the same concentration. The results are shown in table 7 and 8 respectively.

Table 2: Organoleptic characteristics of Partha arishta

Parameter	Description
Colour	Brown
Odour	Alcoholic
Taste	Sweet, astringent
Appearance	Clear

Table 3: Physico chemical evaluation of Partha arishta

Parameter	Observed value
Methanol	Nil
pH	4
Alcohol content (% w/w)	8
Total solid content (% w/w)	7
Specific gravity	0.9970

Table 4: Physico chemical evaluation of Arjuna ghrita

Parameter	Observed value
Acid value	1.45
Saponification value	182
Rancidity	Nil
Specific gravity	0.827

Table 5: Nitric acid scavenging activity of formulations

Sample (µl)	Absorbance (546nm)	% inhibition
Control	0.238	
S1 (Partha arishta)		
12.5	0.171	28.15
25	0.153	35.71
50	0.143	39.91
100	0.127	46.63
200	0.122	48.73
S2 (Arjuna ghrita)		
12.5	0.156	34.45
25	0.138	42.01
50	0.127	46.63
100	0.123	48.31
200	0.117	50.84

Table 6: Nitric acid scavenging activity of standard-Gallic acid

Sample (µl)	Absorbance (546nm)	% inhibition
Control	0.238	
12.5	0.12	49.57
25	0.10	57.98
50	0.09	62.18
100	0.08	66.38
200	0.02	91.59

Table 7: DPPH scavenging activity of formulations

Sample (µl)	Absorbance (517nm)	% inhibition
Control	0.903	
S1 (Partha arishta)		
1.25	0.830	8.08
2.5	0.737	18.38
5.0	0.704	22.03
10	0.652	27.79
20	0.611	32.33
S2 (Arjuna ghrita)		
1.25	0.512	43.30
2.5	0.410	54.59
5.0	0.331	63.34
10	0.319	64.34
20	0.256	71.65

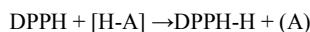
Table 8: DPPH scavenging activity of standard-Ascorbic acid

Sample (µl)	Absorbance (517nm)	% inhibition
Control	0.903	
1.25	0.308	65.89
2.5	0.102	79.00
5.0	0.084	90.96
10	0.072	92.02
20	0.061	93.24

DISCUSSION

Ayurveda is one of the most ancient systems of medicine known today developed through daily life experiences with the mutual relationship between mankind and nature⁷. Ayurveda comprises of various types of formulations including Arishta and Ghrita. In the present study, two Ayurvedic formulations, Arjuna arishta and ghrita were prepared by traditional Ayurvedic methods. Preparation of decoction is the step of prime importance in the manufacturing of arishta formulations. Continuous mild heating during the preparation of decoction facilitates extraction of water soluble components of the herbal drug. But excessive heating results in charring of plant drug. Added *Madhuca indica* flower, *Vitis venifera* fruits and jaggery are source of nutrient and also initiator of fermentation process. *Woodfordia fruticosa* flower has been used as source of yeast. In case of ghrita formulation, the drug material is cooked in water and re cooked in ghrita. Continuous mild heating and stirring during the preparation of ghrita is necessary. Evaluation of organoleptic and physico chemical characteristics of prepared arishta revealed that the formulation is palatable to use and its sweet taste combined with fine aroma which masks unpleasant taste and odour of added herbal ingredients. Self generated alcohol itself act as preservative and improve stability of formulation, no additional preservative is essential. The evaluation of Arjuna ghrita also shows it is a good product. The *in vitro* anti oxidant activity of the formulations were evaluated by nitric oxide scavenging activity and DPPH assay. Nitric oxide has also been involved in a variety of biological functions, including neurotransmission, vascular homeostasis, antimicrobial, and antitumor activities. Despite the possible beneficial effects, its contribution to oxidative damage is also reported⁸. The procedure of nitric oxide scavenging activity is based on the principle that, the sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated by using Griess reagent. Scavengers of nitric oxide compete with oxygen that leads to reduction of nitrite ions production. Scavenging of DPPH free radical is the basis of a

common antioxidant assay⁹. In case of DPPH assay, 1, 1-diphenyl-2-picryl hydrazyl is a stable free radical with red colour which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as,



Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds in terms of hydrogen donating ability. In the present study, both Arjuna arishta and Arjuna ghrita formulations showed a significant antioxidant activity in both methods of evaluations comparing with the standards used.

CONCLUSION

In the present study, two Ayurvedic formulations, Arjuna arishta and Arjuna ghrita were prepared as per traditional methods. The physico-chemical evaluation of these formulations revealed that they are palatable to use. In vitro antioxidant evaluation by nitric oxide scavenging activity and DPPH assay revealed that both the formulations had a significant antioxidant activity. But comparing with arishta, the ghrita formulation showed a better activity in both methods of evaluation. From these results it is clear that further works needed to be done. In the future evaluation of acute toxicity, in vivo antioxidant activity and cardio tonic activity of these formulations may give more valuable results.

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