Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 3, 2013

Research Article

IN VITRO FREE RADICAL SCAVENGING POTENTIAL OF COMMON TRADITIONAL AYURVEDIC EXTRACT: *KASHAYA* AND *KSHEERPAKA*

PALLAVI GHADGE[1], SHITAL GIRAMKAR[1], MEGHA SANGWAN[2], DR. OMKAR KULKARNI[1], DR. ASMITA WELE[2], DR. ANIKET KUVALEKAR[1], DR. SURESH JAGTAP[1], DR. SWATI GADGIL[2*]

[1]Interactive Research School For Health Affairs, Bharati Vidyapeeth Deemed University, Pune 411043, Maharashtra, India, [2]College of Ayurveda, Bharati Vidyapeeth Deemed University, Pune 411043, Maharashtra, India. Email: vd.swatigadgil@gmail.com

Received: 17 Apr 2013, Revised and Accepted: 03 Jun 2013

ABSTRACT

Objective: In *Ayurvedic* therapeutics, drugs in crude as well as processed forms are used. The ingredients are extracted from the plant and put to therapeutic use through various ways; the commonest are *Kashaya* and *Ksheerapaka*. Present study was designed to evaluate comparative free radical potential of these dosage forms.

Methods: *Kashaya* are decoctions prepared using raw herbal material concentrated in water whereas in *Ksheerpaka* milk is used as a solvent for the medicinal components to be extracted. Apart from therapeutic action of the drug, *Ksheerpaka* are supposed to nourish tissues and have more anabolic effects than their respective *Kashaya*. DPPH, ABTS, FRAP and total phenolic content assays of Arjuna, Guduchi and Pipali were performed using the standard procedure.

Results: The percentage inhibition from Fe^{3+} to Fe^{2+}, in case of *Kashaya* was higher (50.29 to 89.92) than respective *Ksheerpaka* (13.65 to 46.79). Similarly, *Kashaya* had comparatively higher DPPH free-radical scavenging activity than *Ksheerpaka* with percentage inhibition of 32.67 to 85.49. ABTS showed very less antioxidant activity in *Ksheerpaka*. The total phenols in *Ksheerpaka* showed undetectable content as against in case of their *Kashaya*. *Arjuna* showed maximum amount of total phenols 56 mg/g of GAE (Gallic Acid Equivalent) than other herbs in comparison.

Conclusion: In contrast to the popular belief in Ayurvedic practice, our studies suggested that *Kashaya* exhibiting better free radical scavenging activity and phenol content than *Ksheerpaka* which can be attributed to the time taken for extraction of contents.

Keywords: *Kashaya*, *Ksheerpaka*, Antioxidant, Arjuna, Guduchi and Pipali.

INTRODUCTION

The use of natural substances as a source of medicines traces back to *Vedic* era. Thousands of herbs are being used for the primary healthcare needs and as nutritional supplements. Indian traditional science, *Ayurved* has a detailed enlistment and explanation of these uses. The medicinal value of the plant is mainly due to the active metabolites and is generally used in the form of an extract where the active constituents are concentrated. This helps to reduce dose of drug, potentiates action and facilitates palatability.

Extraction involves separation of medicinally active portions of plant by using selective solvents in standard extraction procedures [1]. So the products obtained from plants are relatively pure liquids, semisolids or powders intended for internal or external use. These include classes of preparations known as decoctions, infusions, liquid extracts, tinctures, semisolid extracts and powdered extracts. The classical Ayurvedic methods of preparation are complex, tedious and crude. Shortcuts in these preparations may lead to a significant difference in the efficacy and safety of the resultant product. The traditional methods used to prepare Ayurvedic drugs are based on the principles of extraction, concentration and purification [2]. The choice of preparation method depends on the part of the plant to be used, its condition (fresh or dried) and on the drug's expected use.

Based on the basic principles of formulation preparation in *Ayurved*, we have selected the most commonly practiced and the underexplored formulations viz *Kashaya* – water decoctions and *Ksheerapaka* – Milk extracts. These *Kashaya* are mainly prepared by boiling the plant material in specified quantity of water till the active ingredients are extracted. This liquid is then strained through a muslin cloth and is used fresh. The objective of a decoction is to extract the soluble constituents contained within the tough, fibrous cell walls of roots, barks, seeds etc. Decoctions can be used both internally and externally [3]. *Ksheer* i. e. Milk is widely used from ancient era as a food and base of medicament. It has a high nutritive and medicinal value because of its components like proteins, lipids, fatty acids, vitamin, enzymes and minerals which are easily acceptable by healthy individuals as well as patients. Qualities of milk have been potentially used as a medicine by combining it with different herbs as in the case of *Ksheerpaka*. Physical properties of milk substantiate this concept. It has been studied that on gradual increase in the temperature of milk, solubility of fats and proteins also increases, which may enhance the extraction of the medicinally important active constituents [4].

Although *Kashaya* and *Ksheerapaka* from Ayurvedic point of view fall under the same category of decoction, a significant difference in their therapeutic use is assumed. Ayurvedic fraternity uses *Ksheerapaka* when nourishment too is expected along with medication. The question here is whether *Ksheerapaka* is better to *Kashaya* on account of presence of milk or vice versa? So far, in this regards scientific evidences of controversial nature are published. Some of the studies suggested more free radical scavenging potential of *Kasheerapaka* supporting traditional belief and on the other hand some studies have strongly put other verdict [5, 6]. To verify this, we designed an experiment on elucidating comparative free radical scavenging potential of *Kashaya* and *Ksheerapaka* of three commonly practiced herbs namely Arjuna, Pipali and Guduchi.

MATERIALS & METHODS

Plant Material

Dried bark of Arjuna (*Terminalia arjuna* (Roxb.) Wight & Arn.; Family – Combretaceae), stem of Guduchi (*Tinospora cordifolia* (Wild.) Mires ex Hook.f. & Thoms.; Family - Menispermaceae) and fruits of Long pepper (*Piper longum* L.; Family – Piperaceae) were used for study. Plant materials were collected from Pune forest area and authenticated. Their voucher specimens were deposited in herbaria of Medicinal Plants Conservation Centre (MPCC). *Terminalia arjuna* (Roxb.) Wight & Arn. (MPCC 0158); *Tinospora cordifolia* (Wild.) Mires ex Hook.f. (MPCC 3464); *Piper longum* L (MPCC 2330).

Chemicals

All the chemicals and solvents used for the study were of analytical grades. DPPH (2, 2-diphenyl-1-picrylhydrazyl), TPTZ (2, 4, 6-tripyridyl-s-triazine), ABTS (2, 2'-azinobis 3 ethylbenzothiazoline-6-sulfonate), and Trolox were procured from Sigma-Aldrich, USA. Potassium persulfate ($K_2S_2O_8$), Ferric chloride ($FeCl_3 \cdot 6H_2O$),

Hydrochloric acid (HCl), Ferrous sulphate ($FeSO_4$), Folin-ciocalteau, Glacial acetic acid, Sodium acetate ($Na\text{-}CH_3COOH.3H_2O$) Trichloroacetic acid and Methanol were procured from Qualigens Pvt. Ltd, Mumbai, India. Sodium carbonate (Na_2CO_3) and Gallic acid was obtained from SRL, India.

Sample Preparation

Both the Ayurvedic formulations were prepared as per Standard methodology [7, 8].

Kashaya: 20gm plant material soaked overnight in 320 ml water. It was boiled on low flame and reduced to 1/8th of total volume. It was then filtered through muslin cloth, centrifuged and supernatant was used for further analysis. 10mg/ml (w/v) stock solution was made on the basis of sample yield.

Ksheerpaka: 5gm plant material soaked overnight in mixture of 40 ml milk and 160 ml water. It was boiled on low flame till water gets evaporated (1/4th of total volume). It was then filtered through muslin cloth, centrifuged and supernatant was used for further analysis.

1ml of 10% TCA was added in 10 ml *Ksheerpaka* and centrifuged till casein was separated. From supernatant prepared 10 mg/ml (w/v) stock solution was made on the basis of sample yield.

Control: 40 ml milk and 160 ml water was boiled till water gets evaporated. Supernatant was separated after centrifugation. 1ml 10% TCA was added in 10 ml supernatant and used as control for analysis.

***In vitro* biochemical Assays**

2, 2-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

DPPH (2, 2-diphenyl-2-picryl hydrazyl), free radical scavenging activity was determined as per methodology used by Brand-Williams *et al.* (1995) [9], Mukherjee *et al.* (2011) [10] method. When antioxidant reacts with DPPH, it is reduced to the diphenyl-picryl hydrazine with a colour change from deep violet to light yellow. This was quantified spectrophotometrically at 517 nm to indicate the extent of DPPH scavenging activity by the plant extracts.

Various concentrations (100 to 1000µg/ml) of samples were prepared using distilled water as a solvent. In 1 ml of sample 5 ml of methanolic DPPH (33 mg/ L) was added, mixture was incubated at 37^{0}C for 30 min. Methanolic DPPH was kept as control.

The radical scavenging activity of the test samples was expressed as percentage inhibition and calculated according to the following formula:

$$\% \text{ scavenging [DPPH]} = [(A0\text{-}A1)/A0] \text{ X } 100$$

Where,

A0 = Absorbance of the control, A1= Absorbance in the presence of samples.

Total antioxidant activity by FRAP

The total antioxidant capacity of the *Kashaya* and *Ksheerpaka* samples was determined using a ferric reducing antioxidant power (FRAP) assay. FRAP assay was carried out by using modified method of Benzie and Strain (1996) [11] and Nur *et al.* (2012) [12]. It is a simple, automated test measuring the ferric reducing ability of antioxidants. At low pH, ferrictripyridyltriazine (Fe III-TPTZ) complex is reduced to an intense blue colour ferrous (FeII) form.

FRAP reagent was prepared freshly, 300 mM/L of acetate buffer, pH 3.6, 20 mM/L of FeCl3·6H2O, and 10 mM/L of 2, 4, 6-tripryridyl-s-triazine made up in 40 mM/L of hydrochloric acid. All three solutions were mixed together in the ratio 10:1:1. 150 µL of plant extract with various concentration (100 to 1000µg/ml) was allowed to react with 2850 µL of FRAP reagent for 30 min in dark. The absorbance of coloured product was measured at 593 nm. The standard curve was prepared between 100 and 1000 µM FeSO4.

The percent of antioxidant was calculated using following formula:

Percent of antioxidant (%) = [(Absorbance of sample − Absorbance of control)/Absorbance of sample] × 100.

ABTS radical scavenging activity

The method of Mukherjee *et al.* (2011) [10] and Dimitrova et al. (2012) [13] was adopted for the determination of ABTS activity of the *Kashaya* and *Ksheerpaka* samples. The assay is based on the decolorization that occurs when the radical cation ABTS+ is reduced to ABTS (2, 2'-azinobis (3 ethylbenzothiazoline-6-sulfonate).

ABTS radical cations (ABTS+) were produced by reacting ABTS solution (7 mM) with 2.45 mM potassium per sulphate in equal proportion. The mixture was allowed to stand in the dark at room temperature for 12-16 hours before use. The solution was then diluted by deionised water to obtain an absorbance of 0.7 units at 734nm. Fresh ABTS+ solution was prepared for each assay. 60µl extract was added to 2940µl of ABTS solution for 6 minute in dark condition. The standard curve was linear between 10µg/ml and 100µg/ml Trolox. The absorbance was read and calculated percentage inhibition using following formula:

$$\% \text{ inhibition} = [(A0\text{-}A1)/A0] \text{ X } 100$$

Where,

A0 = Absorbance of the control, A1= Absorbance in the presence of samples.

Determination of total phenolic compounds

Total phenol content of samples was measured as Gallic acid equivalents as per Giramkar *et al.* (2012) [14] and Demla *et al.* (2012) [15]. Briefly, 5 ml of Folin ciocalteau reagent (1:10 diluted with water) was added to 0.5 ml sample, 4 ml of 1 M Sodium carbonate added. Reaction mixture was incubated in dark at room temperature (15 min) and measuring absorbance at 765 nm. A Gallic acid standard curve was used to measure the phenolic content and was expressed as mg/g of dry mass of Gallic acid equivalents (GAE).

RESULTS

To evaluate antioxidant activities for *Kashaya* and *Ksheerpaka* using ABTS, DPPH, and FRAP assays; three readings were taken to test the reproducibility of the assays and mean of these values was taken for the analysis.

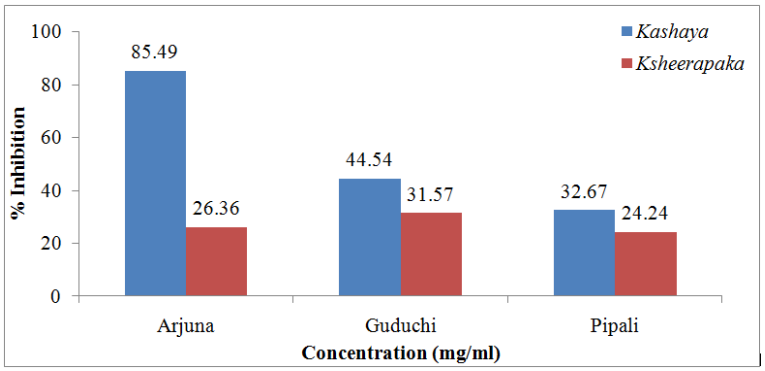

Fig. 1: Percentage scavenging activity of DPPH free radicals by *Kashaya* & *Ksheerpaka*

DPPH Assay

In the DPPH method, the water decoction of Arjuna exhibited potent antioxidant activity with percentage inhibition 85.49, while Guduchi and Pipali showed moderate antioxidant activity with percentage inhibition of 44.54 and 32.67 respectively. Among the *Ksheerpaka,* all 3 samples Arjuna, Guduchi and Pipali exhibited less antioxidant activity which has not differed from Control (Milk). The percentage of inhibitions was ranging from 24.24 to 31.57 (figure 1).

FRAP Assay

In FRAP, *Ksheerpaka* of Arjuna and Pipali showed moderate activity with percentage inhibition of 13.65 and 28.5 respectively, while Guduchi was more active with percentage inhibition of 46.79. The total antioxidant capacity of *Kashaya* of all three plants was significantly greater than *Ksheerpaka*. The percentage inhibition was ranging from 50.29 to 89.92 (figure 2).

ABTS Assay

In ABTS method, *Kashaya* of Arjuna found to be more active as compared to Guduchi and Pipali which exhibited moderate activity. The percentage inhibition was 63.57, 30.74 and 31.01 respectively. While *Ksheerpaka* showed very less antioxidant activity (figure 3).

Total Phenolic Content

Similarly in total phenolic content, *Ksheerpaka* of all 3 samples and *Kashaya* of Pipali showed very less phenolic content. *Kashaya* of Arjuna showed increased amount of total phenols which might have responsible for their higher *in vitro* free radical scavenging activities (Figure 4).

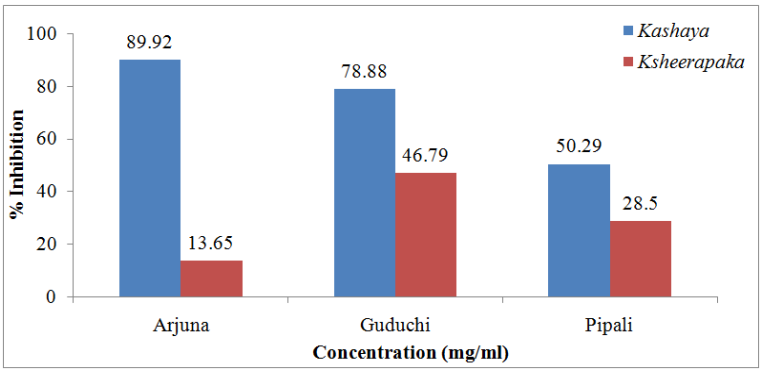

Fig. 2: Comparative FRAP potential of *Kashaya* & *Ksheerpaka*

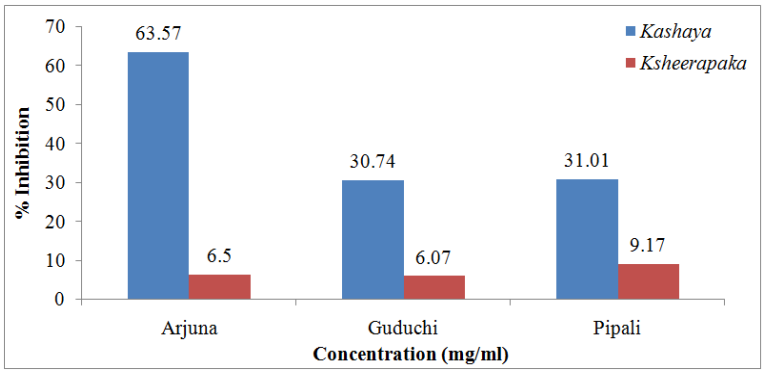

Fig. 3: ABTS radical scavenging activity of *Kashaya* & *Ksheerpaka*

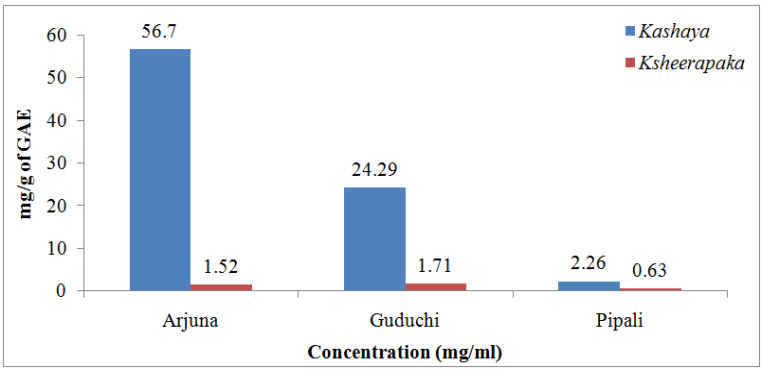

Fig. 4: Total Phenolic Content in *Kashaya* & *Ksheerpaka*

DISCUSSION

The present study analyzed the antioxidant capacity of *Kashaya* and *Ksheerpaka* of 3 different plants namely Arjuna, Guduchi and Pipali used in Ayurveda for treating various diseases. *Kashaya* is a liquid preparation that is made by boiling herb material in water which makes it concentrated. This concentration makes them stronger and thus a smaller dosage is required.

In Ayurveda, for some of the plant drugs *Ksheerpaka* is preferred over *Kashaya* because of their capacity to control *Tikshnata* (piquancy) and bitterness of the drug, to bring palatability in

pediatric patients at times, specific target action and to make it easy to digest. In certain cases, where milk products cannot be prescribed directly but the desired therapeutic efficacies are needed; *Ksheerpaka* may be prescribed as a medicament. It has nourishing property and thus is expected to show immunomodulation. These benefits of *Ksheerpaka* makes it the preferred choice of drug delivery system in aged people and children who have low tolerance to various dosage forms and need added nourishment.

A comparison of *Kashaya* and *Ksheerapaka* for the selected three drugs suggested a significantly higher free radical scavenging potential of *Kashaya* than their respective *Ksheerpaka*. These results are counter stating the earlier studies by Badami *et al* [6] which showed that milk decoction has a higher antioxidant capacity than water decoction as evaluated in the case of coriander, ginger, pepper, tulsi and turmeric.The difference in these findings may be due to the different methods used for extracting sample i.e. we used traditional methods of formulation preparation as against Soxhlet extraction in the above study.

However, our results are in line with the study by Latha *et al* [7] stating that *Kashayas* have better antioxidant protection than *Ksheerpaka* in the case of long pepper, brahmi, liquorice, Ashwagandha and Badam.

Possible reason behind significantly higher free radical scavenging potential of *Kashaya* over their respective *Ksheerpaka* can be attributed to the time taken for extraction of contents. The reduction recommended in preparation of *kashaya* and *ksheerpaka* is 1/8 and 1/4 respectively. This suggests greater extraction of active constituents reflecting into superior results.

CONCLUSION

On the basis of the results obtained in the present study, it is concluded that *Kashaya* of all 3 samples namely Arjuna, Guduchi and Pipali exhibits potent antioxidant and free radical scavenging activities as compared to that of *Ksheerpaka*. However, further studies for developing simpler methods of formulation preparation can be useful to enhance the potential and shelf life of these dosage forms.

REFERENCES

1. Handa SS, Khanuja SPS, Longo G, Rakesh DD. Extraction technologies for medicinal and aromatic plants**.** International centre for science and high technology; 2008. p. 21-54
2. Dunning T. Complimentary therapies and the management of Diabetes and vascular disease. John Wiley and Sons, Ltd; 2006.
3. Savrikar SS, Ravishankar B. Bhaishajya Kalpanaa - The Ayurvedic Pharmaceutics - An Overview. African Journal of Traditional Complementary and Alternative Medicines 2010; 7(3): 174–184.
4. Vaghamshi R, Jaiswal M, Prajapati PK. Ksheera Paka Kalpana - A unique dosages form for special group of patients. Indian Journal of Ancient Medicine and Yoga 2009; 2(2): 89-129.
5. Badami S, Sangeetha M, Latha V, Archana N, Suresh B. Antioxidant potential of five *Ksheerpaka*'s and *Kashaya*'s, Ayurvedic decoctions. Indian Journal of Traditional Knowledge 2007; 6(3): 423-425.
6. Latha TM, Badami S, Banu SS. In vitro antioxidant activity of five Ksheerapaka's and Kashaya's. International Journal of PharmTech Research 2010; 2(3): 1836-1838.
7. Sarngadhracharya P. Sarngadhara samhita with commentary. 3rd ed. Chaukhambha Orientalia, Varanasi; 1983. p. 144
8. Sarngadhracharya P. Sarngadhara Samhita with commentary. 3rd ed. Chaukhambha Orientalia, Varanasi; 1983. p. 167
9. Brand-Williams W, Cuvelier M, Berset C. Use of a free radical method to evaluate antioxidant activity. Lebensm Wiss Technology 1995; 28: 25-30.
10. Mukherjee S, Pawar N, Kulkarni O, Nargarkar B, Thopte S, Pawar P et al. Evaluation of free-radical quenching properties of standard Ayurvedic formulation Vayasthapana Rasayana. BMC Complementary and Alternative Medicine 2011; 11(38): 1-6.
11. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical Journal of Biochemistry 1996; 239: 70-76.
12. Nur FO, Siti AH, Umi KY, Nur APA, Puteri EMW, Uma RS. Phenolics, Flavonoids, Antioxidant Activity and Cyanogenic Glycosides of Organic and Mineral-base Fertilized Cassava Tubers. Molecules 2012; 17: 2378-2387.
13. Dimitrova DZ, Obreshkova D, Nedialkov P. Antioxidant activity of *Tribulus terrestris* – a natural product in infertility therapy. Int J Pharm Pharm Sci 2012; 4(4): 508-511.
14. Giramkar S, Ligade G, Narkhede A, Mukherjee S, Jagtap S, Kulkarni O. et al. Effect of pre – sterilization on physicochemical parameters and *in vitro* free radical scavenging potential of *Saraswatarishta.* Journal of Pharmacy Research 2012; 5(5): 2657-2663.
15. Demla M, Verma H. *In Vitro* antioxidant activity, total phenolic and total flavanoid content of different extracts of *Solanum xanthocarpum* berries. Int J Pharm Pharm Sci 2012; 4(4): 154-157.