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in vitro antioxidant activity of five Ksheerapaka's and Kashaya's

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Abstract: In ayurveda, for several of the crude drugs, milk decoctions are preferred to water decoctions. The milk decoctions are called as ksheerapakas and water decoctions are called as kashayas. The commonly used crude drugs for these preparations includes long pepper, brahmi, liquorice, aswagandha, badam, etc. these preparations are commonly being prepared in homes and regularly feeded to treat / prevent certain diseases. In order to understand the logic behind the use of ksheerapakas for these crude drugs, in the present study a comparative evaluation of ksheerapakas and kashayas was carried out for crude drugs mentioned above. The evaluation of antioxidant activity was done by using standard methods. In ABTS method, water extract of all the five crude drugs showed potent antioxidant activity. Among the milk extracts the badam and long pepper showed moderate antioxidant activity. In the DPPH method, the water extracts of brahmi and liquorice exhibited moderate antioxidant activity. Among the milk extracts long pepper and badam exhibited moderate antioxidant activity. These results clearly indicate that the water extracts have potent antioxidant activity compared to those milk extracts.

Keywords: Ayurveda, Ksheerapaka, Kashaya, antioxidant, free radical.

1. Introduction

The Indian system of medicine itself is of great antiquity and is believed to be one of the most ancient[1]. Extraction is one of the most crucial points in the analytical chain, in the effort of achieving a complete recovery of targeted compounds. In Ayurveda, for several of the crude drugs, milk decoctions are preferred over water decoctions. The milk decoctions are called as Ksheerapakas and water decoctions are called as Kashayas[2]. Usually Ksheerapakas are commonly prepared in homes and regularly feeded especially during the rainy seasons. There is a common belief that the administration of Ksheerapaka prevents many diseases or disorders associated with the arrival of rain like cold, cough etc. Milk being fatty in nature can extract some of the non polar phytoconstituents along with the polar ones. Wherever, Ksheerapakas are preferred, the non polar constituents may be more active therapeutically. In order to understand the reason behind the preferential use of Ksheerapakas over their corresponding Kashayas for some of the crude drugs, the present study was carried out. A comparative evaluation of *in vitro* anti oxidant activity of Ksheerapakas and Kashayas for five crude drugs was carried out. Antioxidant activity was selected due to the involvement of free radicals in the cause of a large number of diseases including cancer, liver disorders, neurological disorders, rheumatoid arthritis, and immunological incompetence etc[3, 4]. Modification in our diet with increased intake of antioxidants may also prove to be effective in decreasing the incidences of these diseases[5].

2. Materials and Methods

2.1. Chemicals

ABTS (2, 2'-azino bis (3-ethyl) benzothiazoline -6-sulphonic acid) and DPPH (1, 1-diphenyl -2-picryl hydrazyl) were obtained from Sigma Chemicals Co., St. Louis, USA. All the chemicals used were of analytical grade.

2.2. Plant Materials Collection and Authentication

The leaves of *Centella asiatica* (Brahmi, apiaceae), roots of *Glycyrriza glabra* (Liquorice, leguminosea) and *Withania somnifera* (Aswagandha, solanaceae) ripe seeds of *Prunus amygdalus* (Badam, rosaceae) and ripe fruits of *Piper longum* (Long pepper, piperaceae) were selected for the study[6, 7]. The dried materials were collected during the month of December – 2005 from the Municipal market, Ootacamund. They were authenticated by Dr. S. Rajan, Medicinal Plants Survey and Collection Unit, Government Arts College, Ootacamund.

2.3. Extraction

The Plant materials were powdered and extracted separately using distilled water and milk. Each plant powder was weighed accurately (1 g) and extracted by heating under reflux for 1 hr with distilled water (100 ml) and milk (100 ml) separately. The extracts were filtered, cooled and made up to 100 ml with the respective solvents and used for the *in vitro* antioxidant studies using the following methods, which are widely used to screen antioxidant properties.

2.4. Scavenging of ABTS radical cation

This method involves the scavenging of ABTS radical cation. The principle behind the technique involves the reaction between ABTS and potassium persulphate to produce the ABTS radical cation, a blue green chromogen. The presence of antioxidant-reductant, the colored radical is converted back to colorless ABTS, the absorbance of which is measured at 734 nm [8, 9].

To 0.4 ml of the extracts or standards added 2.0 ml of distilled DMSO and 0.32 ml of ABTS solution (2 mM, 50 ml mixed with potassium per sulphate 17 mM, 0.3 ml). Absorbance was measuring spectro photometrically after 20 min at 734 nm against the corresponding blank solutions. The assay was performed in triplicate[10]. The percentage inhibition was calculated using the following formula, Inhibition Percentage = [(AC-AA) / AC] x 100, where AC is an absorbance of control and AA is of test solution.

2.5. Scavenging of DPPH free radical

Antiradical activity of the decoctions was tested by their ability to bleach the stable DPPH radical. To 3 ml of DPPH solution (100 mM) in methanol, 0.15 ml of each of the test sample was added and incubated at 37 °C for 30 min[11]. The absorbance was measured at 490 nm against the corresponding blank solutions. The percentage inhibition was calculated[12].

3. Results and discussion

In the ABTS method, the distilled water extracts of all the five plants showed potent antioxidant activity with percentage inhibition ranging from 72.66 to 78.35. The milk extracts of the Badam and Long Pepper showed moderate antioxidant activity with percentage inhibition of 34.5 and 21.4 respectively. The rest of the milk extracts were found to be inactive. The standard ascorbic acid showed an IC_{50} value of 11.25 ± 0.49 µg/ml.

In the DPPH method, the distilled water extracts of Brahmi and Liquorice exhibited moderate antioxidant activity. The percentage of inhibition was ranging from 31.80 to 58.74. The remaining two water extracts were found to be inactive. Among the milk extracts Long pepper and Badam exhibited moderate antioxidant activity with the percentage inhibitions of 48.07 and 51.3, respectively. The remaining three plant extracts were found to be almost inactive. The water extracts of long pepper, brahmi and liquorice were found to be more active than their corresponding milk extracts.

Table 1 – *In vitro* antioxidant activity of five milk and water decoctions

Sample	Percentage inhibition by method*			
	ABTS		DPPH	
	Milk	Water	Milk	Water
Brahmi	-	76.37 ± 1.872	14.31 ± 4.520	55.75 ± 1.639
Liquorice	-	77.77 ± 0.290	4.63 ± 3.404	31.80 ± 4.230
Aswagandha	4.43 ± 1.682	73.73 ± 0.285	2.54 ± 1.951	-
Badam	34.50 ± 1.140	78.35 ± 0.347	48.07 ± 7.870	-
Long pepper	21.40 ± 4.135	72.66 ± 0.360	51.30 ± 8.546	58.74 ± 1.943

* Average of three determinations

4. Conclusion

Peroxide of biological system is regarded to be associated with a number of pathological manifestations. Lipid peroxidation *in vivo* destroys biological membranes leading to change in fluidity and permeability. Hence, free radical mediated processes are implicated in pathogenesis of variety of diseases. Protective role of antioxidants in free radical mediated toxicities is now well established. A large number of plants have shown potent antioxidant activities. Hence, we were interested to screen long pepper, liquorice, aswagandha, badam and brahmi for their possible antioxidant potential using standard *in vitro* methods.

In ayurveda for some of the crude drugs milk decoctions, called as ksheerapakas are preferred over their water decoctions known as kashayas. In present study, a comparative evaluation of antioxidant potentials of both milk and water extracts was carried out for the crude drugs.

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Based on these results, it can be concluded that, in the selected crude drugs the distilled water extracts have potent antioxidant activity, compared to their corresponding milk extracts. It indicates that the polar constituents of these plants possess potent antioxidant activity. Hence Kashayas should be preferred for these crude drugs over Ksheerapakas. However, further studies are required to prove the same. Many polar phenolic constituents like flavonoids and tannins are known to possess strong antioxidant activity[13]. The observed antioxidant activity may be due to the presence of any of these constituents.

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