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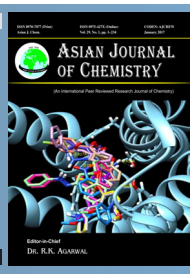

Effect of Drying on Chemical Composition and Antioxidant Activity of Fruits of *Momordica dioica* (Kankoda)

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The fruit of *Momordica dioica* (Kankoda) is a seasonal vegetable and its availability is limited to certain pockets of the country. In order to make it available throughout the year in adequate quantity, these are used in dried form. So our aim was to study the effect of drying on chemical composition and antioxidant activity of fruits of Kankoda. In the present study, fresh and dried fruits were extracted with acetone, ethanol and water separately and the extracts were evaluated for total phenolics, flavonoids and ascorbic acid content on dry mass basis. Their antioxidant activities were determined by the 2,2'-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) free radical scavenging method, β-carotene bleaching method (BCBT) and ferric thiocyanate (FTC) method. Total flavonoids (3.81 ± 0.20 mg CAE/g) and ascorbic acid (0.63 ± 0.01 mg/g) were highest in acetone extract while total phenolic content (5.45 ± 0.26 mg GAE/g) was highest in the water extracts of fresh fruits. Total phenolics (3.58 ± 0.09 mg GAE/g), flavonoids (4.22 ± 0.13 mg CAE/g) and ascorbic acid (0.35 ± 0.01 mg/g) were highest in acetone extract of dried fruits. The flavonoids increased in the acetone extract of dried fruit as compared to the fresh fruit. The antioxidant activity of ethanol extract by DPPH method was found to be maximum (87.02 ± 0.04 %) while in ferric thiocyanate method and β-carotene bleaching method, it was maximum for acetone extract (65.36 ± 1.28 %) and (70.43 ± 0.75 %), respectively for the extracts of fresh fruits. The antioxidant activity of the acetone extract of dried material was maximum (89.03 ± 0.75 %) according to DPPH method and (75.21 ± 1.12 %) by β-carotene bleaching method respectively. Out of these three extracts with acetone, ethanol and water the acetone extracts in general showed maximum antioxidant activity and can be used as a source of antioxidant in foods and medicines.

Keywords: *Momordica dioica*, Phenolics, Flavonoids, Ascorbic acid, Antioxidant activity.

INTRODUCTION

Recent years have shown increasing interest in plants as nutraceuticals and has been focussed on the adoption of crude extract of plants and the possibility that the impact of several diseases may be prevented by intake of phytochemicals and natural nutrients with antioxidant properties. Crude extracts of fruits, herbs, vegetables and other plant materials rich in phenolics can be used in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. The phenolics inhibit the oxidation of macromolecules and thus reduce the degenerative diseases [1].

Momordica dioica Roxb. Ex. Wild. (Cucurbitaceae) known as Kankoda is a perennial, dioecious climber with tuberous roots found throughout India from Himalayas to Ceylon, up to an altitude of 1,500 m. The fruits are used as vegetables and also used in the treatment of mental and digestive disorders [2]. The average nutritional value per 100 g edible fruit was found to contain 7.7 g carbohydrate, 3.1 g protein, 3.1 g fat, 3.0 g fiber and 1.1 g minerals and rest as moisture. It also contained small quantities of essential vitamins like ascorbic acid, carotene, thiamine, riboflavin and niacin [3]. From Kankoda fruit, 8-methyl hentracont-3-ene, pleuchiol, urs-12, 18-dien-3-ol, β-sitosterol, saponin, hederagenin, oleanolic acid, spiranosterol and gypsogenin have been reported [4-7]. Survey of the literature revealed that no work has been done on the phytochemical composition and antioxidant activities of fruits of Kankoda. In the present study total phenolics (TPs), flavonoids, ascorbic acid and antioxidant activities of fruits of Kankoda have been studied. The effect of drying on phytochemical composition and its antioxidant potential were also compared.

EXPERIMENTAL

Fresh fruits of Kankoda were procured from local market at Hisar during July-August, 2012. These were cleaned with water and external moisture wiped out with a dry cloth. The cleaned fruits were chopped into thin slices and extracted separately with acetone, ethyl alcohol and distilled water. 500 g chopped samples in triplicate were shade dried for 4-days.

Then they were placed in oven for further drying at 55 °C for 2 days. These dried fruit slices were then crushed in a grinder into fine powder form and extracted separately with acetone, ethyl alcohol and distilled water. These extracts were then used for determination of total phenols, flavonoids, ascorbic acid and the antioxidant activity using various methods.

The chemicals used were from Sigma-Aldrich, Qualigens, Merk and Ranbaxy of high purity, were used for various experimental procedures.

Preparation of the extracts: 500 g fresh and 40 g dried fruit samples each were extracted separately using acetone, ethyl alcohol and distilled water by refluxing for 6 h and the process was repeated three times followed by filtration and concentration under reduced pressure.

Determination of total phenolics content: The total phenolics were determined by Folin-Ciocalteu reagent method [8] using gallic acid as standard for which a standard curve was prepared using solutions of 0.1, 0.08, 0.06, 0.04, 0.02 and 0.01 mg/mL of gallic acid. A 1 mL of diluted extract, 1 mL of 1 mol/L Folin-Ciocalteau reagent and 2 mL of Na_2CO_3 (20 % w/v) were mixed and the volume was made to 50 mL. After 8 min, the mixture was centrifuged at 6000 rpm for 10 min. Then the absorbance of supernatant solution was measured at 730 nm using Spectronic 20 (Milton Roy, USA) spectrophotometer against a blank prepared similarly with the same reagent but omitting the extract. The amount of total phenols were calculated as gallic acid equivalent (mg GAE/g).

Determination of flavonoid content: Flavonoids content of extracts was estimated according to the method as described by Zhishen *et al*. [9]. Briefly, 1 mL of extracts or solution of catechin (0.02, 0.04, 0.06, 0.08 and 0.1 mg/mL) was added to test tubes containing 4 mL of double distilled water. To the mixture was added 0.3 mL 5 % $NaNO_2$. After 5 min, 0.3 mL of 10 % $AlCl_3$ solution was added. Immediately, 2 mL of 1 M NaOH was added and the total volume was made up to 10 mL with double distilled water. The solution was mixed thoroughly and the absorbance of the samples, blank and standard was read at 510 nm using UV visible spectrophotometer. Total flavonoid content was expressed as mg catechin equivalents per gram of the extract (mg CAE/g).

Determination of ascorbic acid content: Ascorbic acid content was determined by titrimetric method [10]. A known weight of the sample was titrated with 2,6-dichloro phenol-indophenol dye. Ascorbic acid reduces the 2,6-dichloro phenol-indophenol dye to a colourless leuco-base and itself gets oxidized to dehydroascorbic acid. The end point is the appearance of pink colour.

2,2'-Diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) free radical scavenging activity: The free radical scavenging activity was determined using the DPPH as described by Hatano *et al*. [11]. The fruit extracts (acetone, ethanol and water extract) 0.3, 0.6, 0.9, 1.2, 1.5 mg was added to 2.5 mL of 2,2'-diphenyl-1-picryl-hydrazyl radical (DPPH$^{\bullet}$: 0.025 g L^{-1} in methanol) and final volume was made to 10 mL with methanol and mixed by vortex for 5 min. The absorbance of the sample was measured at 517 nm every 10 min till a steady state is reached (2 h) using spectrophotometer. Similarly, a control sample was also prepared. All tests were performed three times. The DPPH radical scavenging effect was calculated as:

Calculation: The percentage of DPPH, which was scavenged (% DPPH*$_{sc}$) was calculated as:

$$\% \text{ DPPH}^{*}_{sc} = \frac{(A_{cont} - A_{samp})}{A_{cont}} \times 100$$

where A_{cont} is the absorbance of control and A_{samp} is the absorbance of sample.

Ferric thiocyanate (FTC) method: The ferric thiocyanate method described by Kikuzaki and Nakatani [12] was used to evaluate the antioxidant activity of the extract. Linoleic acid emulsion was prepared by mixing linoleic acid (0.28 g), Tween 20 (0.28 g) and phosphate buffer (50 mL, 0.2 M, pH 7.0). Test samples were prepared in ethanol-water (6:4 v/v). Different extracts of concentration 0.3, 0.6, 0.9, 1.2, 1.5 mg were mixed with 5 mL of linoleic acid emulsion and final volume made to 10 mL with phosphate buffer (0.2 M, pH 7.0) and incubated at 37 °C. The mixture prepared as above without the extract served as control. Aliquots (0.1 mL) were drawn from the incubation mixture at various intervals and mixed with 0.1 mL of 30 % ammonium thiocyanate w/v, 0.1 mL of 20 mM ferrous chloride in 3.5 % HCl and final volume made to 10 mL with 75 % ethanol. Absorbance was measured at 500 nm in 3 min after the colour was developed. In this method the increase in absorbance is due to increase in the concentration of the peroxide formed indicating the lower antioxidant activity of the sample.

Calculation: Antioxidant activity (%) = (1 – increase in abs. of sample/increase in abs. of control) × 100.

β-Carotene bleaching method: The method as described by Hidalgo *et al*. [13] was used to evaluate the antioxidant activity of the extracts. A 1 mL aliquot of β-carotene solution in chloroform (2 mg per 10 mL), 20 mg linoleic acid and 200 mg Tween 20 were mixed in a flask. Chloroform was removed using a vacuum rotary evaporator and then the mixture was diluted with 50 mL of double distilled water to form an emulsion. A 4 mL aliquots of this emulsion were transferred into test tubes, to which were then added 0.3, 0.6, 0.9, 1.2, 1.5 mg extracts in ethanol. A control consisting of 0.2 mL of ethanol and 4 mL of emulsion was also prepared. The test tubes were incubated at 50 °C and the absorbance at 470 nm was measured at intervals of 30 min, until the colour of β-carotene has disappeared from the control tubes. The above mixture without β-carotene served as blank. All determinations were carried out in triplicate.

Calculation: The antioxidant activity (AA) was evaluated in terms of bleaching of β-carotene sing the formula:

$$AA\ (\%) = 100\left(1 - \frac{(A_0 - A_t)}{(A_0^0 - A_t^0)}\right)$$

where A_0 and A_0^0 are the absorbance values measured at zero time of incubation for the test sample and control, respectively and A_t and A_t^0 are the corresponding values at the end of the reaction time.

RESULTS AND DISCUSSION

The results of estimation of total phenols, flavonoids and ascorbic acid in different extracts of fresh and dried fruits of Kankoda and evaluation of their antioxidant activity by using three testing methods: 2,2'-diphenyl-1-picrylhydrazyl (DPPH)

free radical scavenging method, ferric thiocyanate method and β-carotene bleaching method were used.

Moisture content and extract yield: The moisture content in Kankoda was found to be 84.18 ± 0.88 %. Extract yield of fruits in the tested solvents is given in Table-1. The yields of acetone, ethanol and water extract of fresh fruits were 3.34 ± 0.08 %, 3.63 ± 0.14 % and 1.76 ± 0.11 % and that of dried fruits were 4.06 ± 0.12 %, 16.41 ± 0.65 % and 16.47 ± 0.72 %, respectively on dry weight basis.

TABLE-1
EXTRACT YIELD OF DIFFERENT EXTRACTS OF FRESH AND DRIED FRUITS OF KANKODA

Fruit samples	Acetone extract (%)	Ethanol extract (%)	Water extract (%)
Fresh Kankoda	3.34 ± 0.08	3.63 ± 0.14	1.76 ± 0.11
Dried Kankoda	4.06 ± 0.12	16.41 ± 0.65	16.47 ± 0.72

Values are mean of three replicates ± standard error

Total phenolic content: The effectiveness of antioxidants in natural sources is mainly due to phenolic compounds. Total phenolic content of fresh and dried fruit extracts in three solvents varied considerably from 4.10 ± 0.25 mg GAE/g (ethanol extract) to 5.45 ± 0.26 mg GAE/g (water extract) in fresh fruits (Table-2) and 0.67 ± 0.09 mg GAE/g (water extract) to 3.58 ± 0.09 mg GAE/g (acetone extract) in dried fruits (Table-2). There is decrease of 87.4 ± 0.98 % in the total phenol content in the water extract of dried fruits. This is because of enzymatic hydrolysis of polyglycosylated phenols and aglycones, which gets free from sugar moiety and ester linkages. The free aglycones become more soluble in acetone and less soluble in water, hence its concentration increases in the acetone extract and the order of phenolic content becomes acetone extract > ethanol extract > water extract.

Flavonoids content: Flavonoids are effective radical scavengers [14] and as metal chelators [15]. In fresh fruits of Kankoda (Table-2) flavonoid content varied from 0.87 ± 0.09 mg CAE/g (water extract) to 3.81 ± 0.20 mg CAE/g (acetone extract) while in the dried fruits it was (0.33 ± 0.03 mg CAE/g) in water extract and (4.22 ± 0.13 mg CAE/g) in acetone extract (Table-2). The higher concentration of flavonoids in the acetone extract may be explained as on drying the glycosides of flavonoids as well as its esterified form may have been converted into aglycone form which is more extractable in acetone. However the possibility that after drying the matrix might have been changed to make the flavonoids less extractable cannot be ignored. It was reported by Heim *et al.* [16] that the flavonoid glycosides were found to have lower antioxidant activity as compared to their aglycone moity. The reason is attributed to the fact that the glycoside moiety interferes with the coplanarity of the flavonone molecule and decreases the ability to delocalise electrons which results in decrease in antioxidant activity of the flavonoid.

Ascorbic acid content: Ascorbic acid reacts with H_2O_2, O_2, OH radical and lipid hydroperoxide and in plant tissues it protects against oxidative damage from the oxidant metabolites of photosynthesis and aerobic processes [17,18]. Vegetables are poor resources of ascorbic acid as compared to fruits but abundance of vegetables in local diets contributes to a significant portion of ascorbic acid requirement of human body. In fresh fruits of Kankoda, ascorbic acid content varied from 0.43 ± 0.01 mg/g (ethanol extract) to 0.63 ± 0.01 mg/g (acetone extract) (Table-2). In dried fruits it was lowest (0.08 ± 0.01 mg/g) in water extract and highest (0.35 ± 0.01 mg/g) in acetone extract (Table-2). Among the compounds analyzed ascorbic acid was most affected by temperature (Table-3). The results presented are in line with the data obtained by Kabasakalis *et al*. [19].

Antioxidant activity: Since antioxidants act differently towards a substrate [20], hence to obtain a comprehensive information three different model systems were used for the evaluation of the antioxidant activity of different extracts of fresh and dried fruits of Kankoda.

DPPH method: Free radicals are formed during oxidation of unsaturated lipids which affect human health [21]. Antioxidants scavenge these free radicals. 2,2'-Diphenyl-1-picrylhydrazyl radical is a stable nitrogen centred free radical is generally used for evaluation of radical scavenging activity [22,23]. The hydrogen atom transfer or an electron donor reactions of an antioxidant are monitored by a spectrometer (λ_{max} = 517 nm) in methanol. The decrease in absorbance (colour change from purple to yellow) indicates the conversion of DPPH to the formation of non-radical form DPPH-H, which does not absorb at 517 nm.

TABLE-2
TOTAL PHENOLIC, FLAVONOIDS AND ASCORBIC ACID CONTENTS OF DIFFERENT EXTRACTS OF FRESH AND DRIED FRUITS OF KANKODA

Constituents	Fresh fruit of Kankoda			Dried fruit of Kankoda		
	Acetone extract	Ethanol extract	Water extract	Acetone extract	Ethanol extract	Water extract
Total phenols (mg GAE/g)	4.67 ± 0.27	4.10 ± 0.25	5.45 ± 0.26	3.58 ± 0.09	1.11 ± 0.32	0.67 ± 0.09
Flavonoids (mg CAE/g)	3.81 ± 0.20	1.25 ± 0.19	0.87 ± 0.09	4.22 ± 0.13	0.73 ± 0.06	0.33 ± 0.03
Ascorbic acid (mg/g)	0.63 ± 0.01	0.43 ± 0.01	0.47 ± 0.01	0.35 ± 0.01	0.14 ± 0.01	0.08 ± 0.01

Values are mean of three replicates ± standard error; mg GAE/g = milligrams gallic acid equivalent/g of the extract; mg CAE/g = milligrams catechin equivalent/g of the extract

TABLE-3
PERCENT INCREASE (+)/DECREASE (-) IN CONCENTRATION OF VARIOUS CHEMICAL CONSTITUENTS OF FRUITS OF KANKODA ON DRYING

Constituents	Acetone extract	Ethanol extract	Water extract
Total phenols (mg GAE/g)	-23.03 ± 1.32	-73.33 ± 0.87	-87.41 ± 0.98
Flavonoids (mg CAE/g)	+11.38 ± 0.89	-41.44 ± 1.33	-62.25 ± 1.06
Ascorbic acid (mg/g)	-44.66 ± 0.97	-68.26 ± 1.12	-83.41 ± 1.20

$$DPPH^{\bullet} + AH \longrightarrow DPPH\text{-}H + A^{\bullet}$$

The antioxidant potential of the extracts evaluated for radical scavenging activity against $DPPH^{\bullet}$ is shown in Figs. 1 and 2. The antioxidant activity exhibited by acetone, ethanol and water extracts of fresh fruits were as 76.11 ± 0.02 %, 87.02 ± 0.04 % and 58.01 ± 0.05 %, respectively at the concentration of 1.0 mg/mL of extracts (Fig. 1). The corresponding values for dried fruits of Kankoda at the same concentration were 55.51 ± 0.71 %, 85.61 ± 0.95 % and 89.03 ± 0.75 %, respectively (Fig. 2). The higher scavenging activity or water extract in both cases may be due to higher concentration of polyphenols which are more extractable in water.

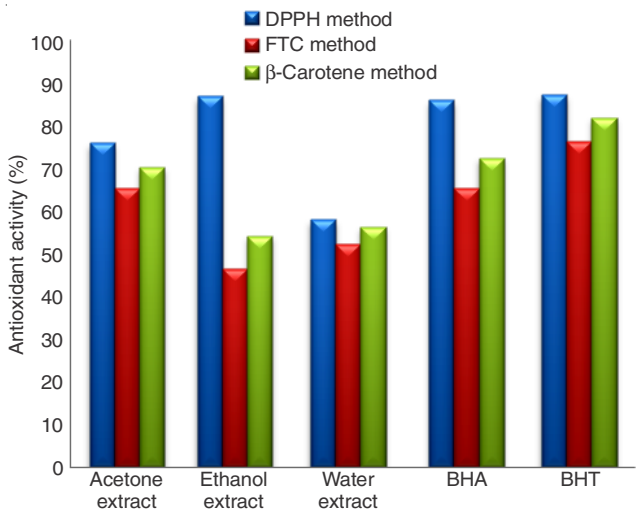

Fig. 1. Antioxidant activity of different extracts of fresh fruits of Kankoda at a concentration of 1.0 mg/mL by DPPH, ferric thiocyanate and β-carotene methods

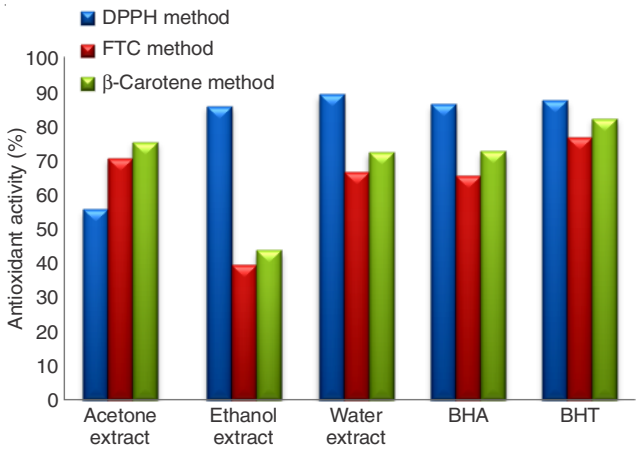

Fig. 2. Antioxidant activity of different extracts of dried fruits of Kankoda at a concentration of 1.0 mg/mL by DPPH, ferric thiocyanate and β-carotene method

Antioxidant activity by ferric thiocyanate method: During the oxidation of linoleic acid at 37 °C, peroxides are formed which oxidizes Fe^{2+} to Fe^{3+} ion [24]. The ferric ion reacts with thiocyanate and forms a blood red coloured ferric thiocyanate which has a maximum absorbance at 500 nm. The antioxidant activity exhibited by acetone, ethanol and water extracts of fresh fruits were as 65.36 ± 1.28 %, 46.41 ± 0.95 % and 52.21 ± 1.12 % at the concentration of 1.0 mg/mL of extracts, respectively (Fig. 1). The corresponding values of dried fruits at the same concentration were 70.46 ± 0.98 %, 39.51 ± 1.22 % and 66.36 ± 1.28 %, respectively (Fig. 2). The results obtained by thiocyanate method supports the results of the β-carotene bleaching method.

Antioxidant activity by β-carotene method: In the β-carotene bleaching test, the linoleic acid free radical formed as a result of abstraction of allylic hydrogen form the radical adducts of carotenoids. The presence of antioxidants by neutralizing the free radical hinder the formation of radical adducts and the loss of characteristic orange colour is monitored spectrophotometrically [25]. Present results showed that the antioxidant activity of fresh fruits (Fig. 1) varied from 54.31 ± 0.79 % (ethanol extract) to 70.43 ± 0.75 % (acetone extract) and from 43.51 ± 1.19 % (ethanol extract) to 75.21 ± 0.97 % (acetone extract) in dried fruits (Fig. 2) at the concentration of 1.0 mg/mL of the extract. BHA and BHT were used for comparative purposes. The highest antioxidant activity of acetone extract in both the fresh and dried fruits can be explained as flavonoids are more extractable in acetone and hence more activity. The water extract of the dried fruit showed higher activity (72.26 ± 1.12 %) as compared to water extract of the fresh fruit (56.31 ± 1.09 %). This may be explained as during drying the phenols and flavonoids, present in the acylated and glycosylated form undergo degradation and release aglycones which are more active than their glycosylated or acylated form. The other reason may be attributed to synergistic effect of antioxidative compounds and with other components of the extract of dried fruit [26].

Conclusion

During drying and also during food processing many changes that could affect antioxidant activity take place. The results indicate that higher amount of phenolics and flavonoids are extracted from the fresh fruits than the oven dried fruits. This may be explained by the decomposition of flavonoid glycosides and esters on storage or under higher temperature The differences found in the antioxidant values may be related to the large qualitative and quantitative differences in the phenolics content The water extracts of the dried fruits of Kankoda showed increase in antioxidant activity in all the three test methods, acetone extract showed increase in the antioxidant activity in ferric thiocyanate and β-carotene bleaching methods. The results in the present study provide the evidence that the dried vegetable have a significant ability to show radical scavenging activity and this suppress lipid peroxidation. Hence, Kankoda can be considered as a useful source of natural antioxidants for human health and it can be used in dried form. The results of the present study corroborate with the findings of a great deal of the previous work in this field.

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