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Value addition scenario of arid foods of desert area and evaluation of their nutritional and phytochemical potential

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Abstract

Desert area is gifted with valuable natural resources particularly arid fruits and vegetables. Arid fruits are used by native people as a prime source of food with some traditional value attached to it. Processing of traditionally important arid foods into more functional and convenient product can improve livelihood security of the people worldwide. Thus, value addition prospects have been studied and reported along with its nutritional composition, phytochemicals and antioxidant activities. The effect of various solvents on extraction procedure and their activities were also analyzed. The study demonstrated Kair and Kachri the arid fruits of Rajasthan to be rich in proteins and carbohydrates.

Key-Words: *Capparis deciduas, Cucumis callosus,* solvent extraction, phenolics and antioxidant activity

Introduction

Metabolic process, UV radiation, environment pollution are fewer causes for generation of free radicals. Action of many carcinogens and mutagens is via free radical generation [1,2]. Use of antioxidants in our diet protect against free radicals. Antioxidants help in scavenging free radicals thus preventing from various diseases[3]. Unsaturated fatty acids present in biomembrane by the attack of free radicals cause lipid per-oxidation thus destruction of proteins and DNA that may lead to various heart diseases, cancer, ageing[3]. Fruits and vegetables are naturally are very good antioxidant source where antioxidant activity is ascribed due to presence of phenolics, flavonoids, vitamins, caretenoids and secondary metabolites[4]. *Capparis decidua* is a medicinal plant, belongs to the family *Capparidaceae*[5] and commonly known as 'Kair' in Hindi. Amino acid composition studies, fatty acid profiling, tocoferols and sterols have been identified in *Capparis decidua* seeds[6]. *Cucumis callosus* (Kachri) belongs to family *Cucurbitaceae.* Both are very important ingredient of royal and famous dish of Rajesthan, India. Aqueous extract of *Cucumis callosus* seeds inhibit free radicals of DPPH[7].

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Thus, the study on *C. deciduas* and *Cucumis callosus* is carried out to characterize various nutritional, phytochemicals and antioxidant activity of Kair and Kachri fruit and their use as a potential source of natural antioxidants in order to understand their nutritional and other health benefits.

Material and Methods

Kair (*Capparis decidua*(Forssk.) Edgew) and Kachri (Cucumis *callosus* (Rottler) Cogn.) dried fruits were collected from Delhi, India and were identified at NISCAIR. The partially crushed samples were used for various parameters.

Solvent extraction

Partially crushed samples were extracted with Ethyl acetate, Methanol, n-Hexane and water. The extracts were dried under vaccum and stored at 4ºC.

Chemical analysis

Macrokjeldhal method was used for estimation of crude protein content[8]. Crushed samples of dried fruits were put in an oven at 105°C for 24 h. Difference in weight determines the moisture content[9]. The ash content was analyzed by AOAC method Ref. 942.05. The fat content of the samples was determined by using PE as a solvent. Crude fiber was determined[10]. Total carbohydrate[9] and Energy calorific value[8] were also calculated.

Phytochemical analysis

Crude alkaloids determination

2.5g sample mixed with 100ml 10% acetic acid in ethanol and incubated for 4h at RT. Sample was filtered and concentrated to ¼ of original volume using

water bath. Concentrated ammonium hydroxide added drop wise to the extract until the precipitation was complete. The precipitate washed with dilute ammonium hydroxide solution and filtered. Crude alkaloid was weighed[11].

Saponins determination

5 g of sample mixed with 50ml of 20% ethanol. The suspension was heated over a hot water bath for 4 h with continuous stirring at about 55°C.The mixture was filtered and the residue re-extracted with another 50ml of 20% ethanol. Combined extracts were reduced to 10 ml over water bath at about 90°C. The concentrate transferred to a separating funnel and 20ml of diethyl ether added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 15 ml of n-butanol was added. The combined n-butanol extracts washed twice with 10ml 5% aqueous sodium chloride. The remaining solution heated over water bath, the samples were dried in the oven to a constant weight. The saponins content was calculated in percentage[12].

Tannin determination

5g of the sample was weighed into plastic bottle. 50ml distilled water was added and shaken for 1h in a shaker. This was filtered into a 50 ml of the volumetric flask and made up to mark. 5ml of the filtrate was pipette out into a tube and mixed with 3ml of 0.1M $FeCl_3$ in 0.1 N HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 605nm wavelength within 10 min. A blank sample was prepared and the color developed and need at the same wavelength. A standard was prepared using tannin acid to get 100 ppm and measured[13].

Total phenolics determination (TPH)

Folin Ciocalteu reagent method was used to determined total phenols[14]. 100µl of sample mixed with 1.150 ml of distilled water and 250µl of FC reagents and vortexed and added 1.5ml of 20% Na_2CO_3. After 2h added 2 ml DW and absorbance was measured at 765 nm. Gallic acid (0-100 µg/ml) was used as standard for preparation of calibration curve. Total phenol values were expressed in terms of gallic acid equivalent (mg g^{-1} of dry extract).

Total flavonoids determination

Aluminum chloride colorimetric method was used for flavonoids determination[15]. 250µl extract mixed with 4.5ml DW and 0.3ml $NaNO_2$ (5 %). After 5 min added 0.3ml of $AlCl_3$ (10%). Added 2ml of NaOH (1M) after 6 min. Volume made upto 10 ml with DW and absorbance was taken at 510 nm. Rutin was used as standard for preparation of calibration curve.

Total flavonol determination

250µl extract mixed with 1ml of ethanol followed by 1ml of 2% Aluminium chloride solution with mixing. 3ml of 5% sodium acetate solution was added and incubated at 20°C for 2.5h. Absorbance was measured at 440nm. Rutin was used as the standard for calibration curve. The flavonol content was expressed in mg of rutin equivalents (RE) per gram of dry weight of sample[16].

Antioxidant Activity

DPPH radical scavenging activity

0.1ml of extract mixed with 1ml of DPPH. Absorbance was measured at 517nm[25] after 30 min of incubation. Ascorbic acid and trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) were used as standards[17].

ABTS assay

10µl of extract was mixed with 990 µl ABTS reagent and incubated for 10 min. Absorbance was measured at 734nm. Ascorbic acid and trolox were used as standards[18].

FRAP assay

100µl of extract was mixed with 900µl of FRAP reagents. Absorbance was measured at 593nm[27] after 4min of incubation at RT. FRAP values were calculated as mg of trolox equivalents/g extract (TE)[19].

Results and Discussion

Chemical analysis

Potential benefits were shown by nutritional attributes of Kair and Kachri (Table 1). In present studies, Kair and Kachri were found to be rich in proteins and carbohydrates. However, crude fat was found to be very low in kair. Moisture content and dry matter analysis reporting during nutritional analysis is very important because it directly affect the nutritional content of the fruits, vegetables. Ash and moisture content significantly vary in both the samples on dry weight basis.

Phytochemical analysis

The phytochemical content of Kair and Kachri were analyzed and the values of tannins, saponins and crude alkaloids were determined on dry weight basis (g/100g) (Table 2). High quantity of alkaloids was found in Kachri. Tannin content was found to be nearly similar in Kair and Kachri. The value of saponins was found to be almost related in both the samples. High amount of alkaloids correspond to spasmolytic and anesthetic agents. Saponins helps in boost of the immune system, lower the cholesterol levels in the blood and reduce the risk of getting intestinal cancer.

Phenolic compounds (Table 3) help in plant defense mechanism by counteracting reactive oxygen species (ROS) in order to prevent molecular damage[20].Total

phenolic content ranged between 49-154 µg GAE/mg extract and 56-72 µg GAE/mg extract in kair and Kachri respectively in different solvent extracts. Research studies have shown that phenolic compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma, and flavor and also in providing health beneficial effects. Phenolics provide plant defense mechanisms to neutralize reactive oxygen species (ROS) in order to survive and prevent molecular damage and damage by microorganisms, insects, and herbivores[20]. Highest phenolic compounds were obtained in n-hexane extracts of Kair which suggest that major compounds are less polar and therefore retrieved in n-hexane. Flavonoids and flavanol content was also found highest in n-hexane extracts only in both kair and Kachri (Table 3).

Antioxidant Activity:

DPPH scavenging activity

DPPH is a free radical generating compound and has been widely used to evaluate the free radical scavenging ability of various antioxidant compounds. Figure 1.shows that with increasing conc. of extracts, % scavenging of DPPH free radicals also increases. The order of antioxidant activity assessed by DPPH was n-hexane>DCM > Acetone>Aqueous (Kair) and MeOH>Ethyl acetate>n-hexane (Kachri). Trolox was used as standard.

ABTS scavenging activity

ABTS scavenging assay is applicable for screening both lipophillic and hydrophilic antioxidants. IC_{50} values of different extracts of Kair and Kachri on ABTS radical is given in Table 3. The order of antioxidant activity assessed by ABTS was- Aqueous > Acetone >DCM > n-hexane (Kair) and MeOH> Ethyl acetate> n-hexane (Kachri).

FRAP assay

Trolox was used as the standard curve for FRAP. Values of Trolox equivalents for the samples were calculated by extrapolation standard curve ($y = 0.011X$, $R^2 = 0.983$). N-hexane extract showed high yield for both the samples whereas DCM extract showed nearly equal values as that of n-hexane in case of Kair (Table 3).

Two important ingredients of Panchkuta were analyzed in terms of nutritional, phytochemicals and antioxidant potential for their use as functional foods and nutraceutical to provide health benefits. Both the ingredients contain appreciable amount of proteins which makes Panchkuta a highly proteinaceous diet. Kair has very low amount of fat which makes it ideal diet for overweight people. It can be used in weight reduction diets also. High amount of alkaloids are present in Kachri fruits so it can be further tested for spasmolytic and anesthetic agents. Both the samples contains high amount of saponins. Saponins helps in boost of the immune system, lower the cholesterol levels in the blood and reduce the risk of getting intestinal cancer. Moderate antioxidant activity was shown by both the ingredients of Panchkuta.

There is a significant traditional insight available on different therapeutic and nutritional uses of arid fruits and vegetables along with great potential in the field of value addition. Therefore, there is a good future scope in functional foods in terms of availability, quality and therapeutic uses of arid fruits and vegetables.

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Table1: Proximate composition of Kair and Kachri (g/100g)

Species	Ash	Moisture	Crude fat	Total protein	Total carbohydrate	Crude Fiber
kair	7.69±0.121	0.9±0.021	0.64±0.021	45.116±0.321	45.654±0.562	0.8±0.021
kachri	11.8±0.160	2.5±0.061	6.828±0.165	23.846±0.991	61.214±0.615	0.23±0.165

Table2: Phytochemical content of Kair and Kachri (g/100g)

Species	Crude alkaloids (g/100g)	Tannins (g/100g)	Saponins (g/100g)
Kair	2.16±0.0355	0.130±0.001	7.80±0.340
Kachri	12.96±0.086	0.177±0.007	7.28±0.077

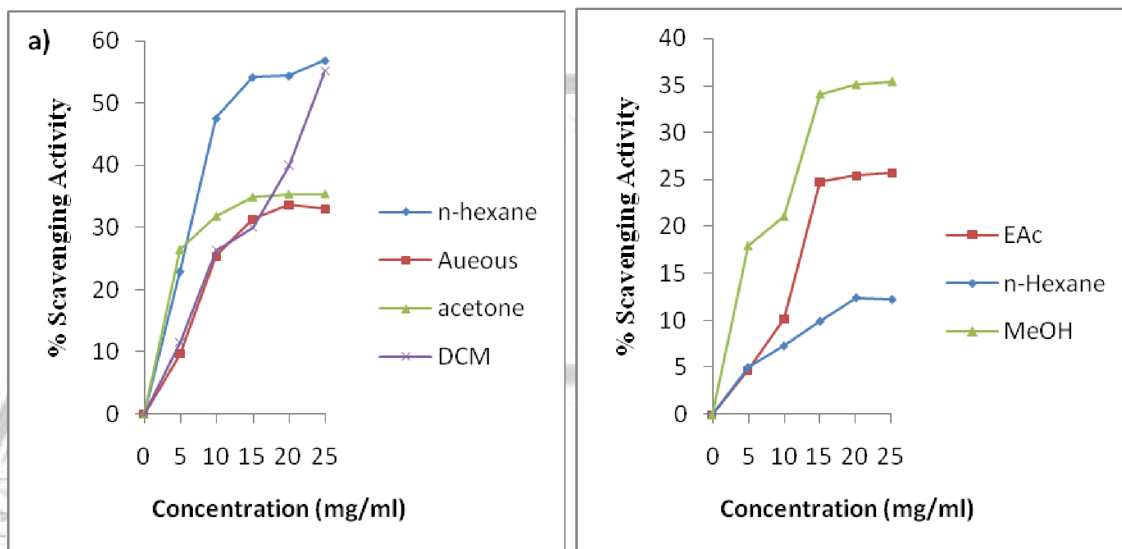

Fig. 1: % Scavenging activity of various extracts of a) Kair b) Kachri

Table3: Phytochemical and antioxidant content in kair and kachri

		Kair				Kachri		
		Aqueous	N-Hexane	DCM	Acetone	Ethyl acetate	n-Hexane	Methanol
Phenolic compounds	Total Phenolic (µg GAE/mg extract)	154 ± 2.041	357 ± 2.094	63.66 ± 2.65	49.83 ± 2.718	56.66 ± 6.78	72.33 ± 7.14	61.66 ± 6.94
	Total Flavonoid (µg RE/mg extract)	98.33 ± 8.49	812.33 ± 9.53	130.33 ± 19	106.33 ± 8.95	240.66 ± 18.2	269.33 ± 6.54	20.33 ± 0.471
	Total Flavanol (µg RE/mg extract)	148.33 ± 20.54	868.33 ± 2.86	776 ± 9.09	341 ± 3.26	681.66 ± 3.29	883.33 ± 2.49	681.66 ± 3.29
Antioxidant activity	DPPH IC_{50} (mg/ml)	N.D.	12 ± 0.458	23± 0.318	N.D.	N.D.	N.D.	N.D.
	ABTS IC_{50} (mg/ml)	9.3 ±0.085	N.D.	28 ± 0.315	16.5 ± 0.396	N.D.	N.D.	61±0.28
	FRAP (mg TE/g extract)	71.42± 4.369	74.33± 0.3858	74.15± 1.78	72.21± 0.171	72.72± 0.598	75.636± 1.2	70.9 ± 1.243

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