ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 8, Issue 5, 2015

ISSN - 0974-2441

Research Article

COMPARATIVE FOURIER TRANSFORM INFRARED SPECTROSCOPIC ANALYSIS AND FREE RADICAL QUENCHING PROPERTIES OF THREE *CASSIA* SPECIES

MAHENDRA S. KHYADE[1]*, SURESH P. KAMBLE[2], ANIL R. KURHE[3], ANUP D. PADWAL[1]

[1]Department of Botany, SN Arts, DJM Commerce and BNS Science College, Sangamner - 422 605, Maharashtra, India. [2]Department of Integrated Biotechnology, School of Life Sciences, SRTM University, Nanded - 431 606, Maharashtra, India. [3]Department of Zoology, Arts, Commerce and Science College, Satral - 413 711, Maharashtra, India. Email: maheshkhyade@gmail.com

Received: 20 May 2015, Revised and Accepted: 11 June 2015

ABSTRACT

Objective: To evaluate the comparative Fourier Transform Infrared spectroscopic analysis and the in vitro antioxidant screening of leaf extract of three Cassia species.

Methods: The 80% leaf methanol extract of all three species was tested for their total phenols, flavonoids, antioxidant screening (DPPH, ABTS, Nitric oxide, radical reducing power and total antioxidant capacity) and along with spectroscopic study.

Results: The results revealed that C. uniflora had the highest total phenolic content (276.54±0.031 µg/ mg DW) followed by C. occidentalis and C. tora, while the highest total flavonoid content (43.71±0.086 µg /mg DW) was also recorded to C. uniflora followed by C. tora and C. occidentalis. FTIR screening revealed the presence of various functional groups such as aldehydes, alcohols, carboxylic acid, alkenes, aromatic compounds, esters, nitro compounds and alkyl halides in all the three species. Overall all the species exhibited dose dependent antioxidant activity in tested in vitro models. The antioxidant activity was varied to each species; the highest IC50 values for DPPH (58.55±0.213 µg/ml) were depicted in C. occidentalis followed by C. tora and C. uniflora. The prominent IC50 values for ABTS radical scavenging was reported in C. uniflora extract (IC50value of 0.074±0.003 µg/ml) followed by C. occidentalis and C. tora (with IC50 values of 24.388±0.183 and 29.388±0.368 µg/ml equivalent to ascorbic acid).

Conclusion: The results of the present study indicate that, of the Cassia species evaluated, C. occidentalis exhibited strong activity followed by C. tora and C. uniflora, and can be used as a potent source of natural antioxidants.

Keywords: *Cassia* species, Leaf extract, Fourier transform infrared spectroscopy, Antioxidant activity, Ascorbic acid.

INTRODUCTION

The genus *Cassia* (family: Caesalpinaceae) represents one of the largest groups of flowering plants, having herbs, shrub along with tree habit and is widely distributed in tropical and subtropical regions of the world [1]. It includes 580 species throughout the world, of which only twenty species are reported in India [2,3].

Cassia occidentalis L. (Syn.: *Senna occidentalis* Roxb.) a diffuse undershrub is known by various names in different language, e.g. The Negro Coffee (English), Kasamara (Sanskrit), Kasondi (Hindi) and Ran tarota (Marathi). It grows in the tropical and subtropical countries, including the United States, Africa, Asia, and Australia [4,5]. In India, *C. occidentalis* commonly grows along the road sides, ditches and waste dumping sites as a common weed [6] and reported for different ailments in indigenous and folk medicines [7-9]. In Ayurveda, the leaves are prescribed for aphrodisiac, cough, asthma, hiccup and fever [10]. Furthermore, it is used as cardiac tonic, ringworm, leprosy, skin disease, and constipation. In folk medicine, the leaf paste is externally applied on healing wounds, sores, itch, and cutaneous diseases [11]. In Benin, the decoctions of leaves are given in fever [12]. West Indian people used its decoction for drinking and applied leaves to the body for fever and Dominicans use leaf tea for colds, cuts, fevers and pregnancy [13]. In Brazilian traditional medicine, leaves have been widely used as a laxative, analgesic, febrifuge, diuretic, hepatoprotective, vermifuge and colagogo as well as for the treatment of tuberculosis, gonorrhea, dysmenorrhea, anemia, flu, liver and urinary tract diseases [14,15]. In Brazil, the preparation made of hydroalcoholic extract of leaf along with stem has been marketed by pharmaceutical laboratory (LAPERLI) with commercial name of Cassia Virgínica® and has been prescribed for the treatment of flu, fever, erysipelas, febrifuge, as an analgesic, hepatoprotective, and diuretic [16]. Previous studies on this species pertaining to phytochemicals have reported the alkaloids, flavonoids, tannins, phlobatannins, chrysophanol, emodin, physcion, tetrahydroanthracene derivatives, germichrysone, and occidentalins A and B in the leaves of *C. occidentalis* [17]. Furthermore, flavonoid such as apigenin, chrysophanol and emodin [18] bianthraquinone 1,1-bi-4,4′,5,5′-tetrahydroxy-2,2′-dimethyl anthraquinone, flavone metterucinol-7-O-α-L rhamnoside [19,20]. The biological activities of *C. occidentalis* leaves demonstrated antimicrobial [21-23], antimalarial [24,25], anti-inflammatory [26], hepatoprotective, and antioxidant potential [1,27,28].

Cassia tora L. (Family-Caesalpiniaceae) in an annual fetid herb, generally distributed in India, Srilanka, West China and tropics. In India, it is commonly seen throughout the country along roadsides and in waste ground in rainy season. Locally, it is known by various names such as Takala (Marathi); Charota (Hindi). In China it called as Jui Ming Zi and Foetid Cassia (English) [29]. The leaves are mentioned in ayurvedic medicine as a laxative, antiperiodic and are useful for leprosy, ringworm, bronchitis, and cardiac disorders [7,30]. It is also used in fever, constipation, as a liver tonic, expectorant and against helminthes [10]. In Africa, the leaves are employed in the treatment of impetigo, ulcers, helminthiasis and as a purgative [31,32]. In addition to this, the powdered leaves are used for ulcers and parasitic skin conditions [33]. Furthermore, the fermented leaves are pounded and added to food as well as in "local gin" (strong liquor) and given orally for anthelmintic and as a purgative effects [31,32]. In Vietnam, the alcoholic or vinegar maceration of pounded leaves is applied externally to treat eczema and dermatomycosis [34]. Literature reveals that many scientific evaluations on leaves of *C. tora* have been carried out. These are anti-inflammatory [35], hepatoprotective [36,37], antinociceptive [38], cytotoxic [39], anticancer [30]. The leaves have reported many constituents such as rich in emodin, tricontan-1-ol, stigmasterol, β-sitosteral-β-D-glucoside, freindlen, palmitic, stearic, succinic and d-tartaric acids, uridine, quercitrin and iso-quercitrin [40-43].

Cassia uniflora Mill. (Syn. *Cassia senna*) is an invasive, erect, fast growing herb, usually found along the roads sides, waste places and has spread everywhere as a common weed throughout India. There is a little literature on the therapeutic application, phytochemistry and antioxidant activity of this species.

There are a few reports on the antioxidant activity of *C. occidentalis* and *C. tora*. This study is an attempt to evaluate the comparative phytochemical profiles and *in vitro* antioxidant activities of methanolic leaf extracts (80%) of *C. occidentalis*, *Cassia tora* and *C. uniflora* growing in Maharashtra, India, in order to discover their potential for free radical scavenging activity.

METHODS

Sample collection and extraction

For this study, the fresh leaves of three *Cassia* species under study, *viz*., *C. occidentalis, C. tora* and *C. uniflora* were collected from different localities of Sangamner tehsil of Ahmednagar district (MS), India. The plant specimens were authenticated at the Postgraduate Department of Botany, Sangamner College, Sangamner, by using Floras [44,45]. The collected plant material was washed with tap water and air dried on the laboratory bench for 15 days and then ground to fine powder using an electric mill. Dried and coarsely powdered leaves (100 g) of all three species were extracted with 80% methanol by cold extraction method [46,47]. The mixture was kept for 24 hrs with frequent shaking at room temperature to allow the extraction of compounds. Extract obtained was passed through filter paper and respective solvent was removed using rotavapor (at 40°C).

Phytochemical screening

Qualitative phytochemical analysis

Extracts of all three *Cassia* species were analyzed to detect the phytoconstituents such as alkaloids, cardiac glycosides, caumarins, flavonoids, reducing sugar, saponins, steroids, tannins, and terpenoids using standard protocols [46,48].

Quantification of total flavonoids

The total flavonoid content of the extracts was determined using prescribed method [49]. In brief; 0.1 ml of sample solution (1 mg/ml) was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5% $NaNO_2$ solution. After 6 minutes of incubation, 0.15 ml of 10% $AlCl_3$ solution was added and then allowed to stand for 6 minutes, followed by adding 2 ml of 4% NaOH solution to the mixture. Immediately after this, water was added to the sample to bring the final volume to 5 ml, the mixture was thoroughly mixed and allowed to stand for another 15 minutes. The absorbance of the mixture was determined at wavelength 510 nm. The total flavonoid content was expressed in microgram of rutin equivalents (RE) per milligrams of extract.

Quantification of total phenolics

The total phenol content of the extracts was analyzed using Folin–Ciocalteu method [50]. In brief, the extracts (0.2 ml of 1 mg/ml) were mixed with 2.5 ml of distilled water, 0.5 ml of the Folin–Ciocalteu reagent and 1.0 ml of Na_2CO_3 reagent were added to the mixture. They were then incubated at room temperature for 30 minutes. The absorbance of the mixture was measured spectrophotometrically (Systronic UV-VIS India) at wavelength 765 nm. The total phenol content was expressed in microgram gallic acid equivalents per milligram of extract. Triplicate measurements were taken and data were presented as mean ± standard deviation (mean±SD).

Fourier transform infrared spectroscopy (FTIR) analysis

FTIR analysis of methanol extract was performed using Chemito 410 spectrophotometer. The extracts was ground into a fine powder using an agate mortar along with a standard KBr pellet and examined with the FTIR spectrometer in the region 4000-400 cm^{-1} and the peak values were recorded [51,52].

In vitro antioxidant activity

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

The DPPH free radical scavenging capacity of extract was measured by using standard protocol [53] with slight modifications. Briefly, the reaction mixture contained 300 µl of extract of varying concentrations (10-300 µg/ml) and 2 ml of DPPH solution. After 10 minutes, the change in absorbance was recorded at 517 nm in a spectrophotometer against a blank. L-ascorbic acid was used as a positive control. The DPPH radical scavenging capacities were expressed as ascorbic acid equivalent antioxidant capacity in m mol/g of extract. The % DPPH scavenging activity was calculated by the equation:

$$\% \text{ DPPH scavenging activity} = A_{control} - A_{sample}/A_{control} \times 100$$

Where $A_{control}$ is the absorbance of the control (DPPH + methanol) and A_{sample} is the absorbance of the sample (DPPH + methanol + sample).

2,2-azinobis [3, ethyl-benzothiazoline-6s-sulphonic acid] (ABTS) radical scavenging assay

ABTS radical scavenging activity was determined by using standard method [54]. The ABTS radical cations are produced when ABTS (7 mM) reacts with potassium persulfate (2.45 mM) when incubated at room temperature in the dark for 16 hrs. The solution thus obtained was further diluted with phosphate buffer saline (PBS) to give an absorbance of 1.000. Different concentrations of the test sample in 50 µl were added to 950 µl of ABTS working solution to give a final volume of 1 ml. The absorbance was recorded immediately at 734 nm. Ascorbic acid was used as a reference standard and inhibiting concentrations of extracts were tested at 10, 20, 40, 60, 80, and 100 µg/ml. Reference standard (ascorbic acid) was also tested with similar concentrations and percent inhibition was calculated by following equation:

% inhibition = ([Absorbance of control – Absorbance of test sample]/ Absorbance) x 100).

Nitric oxide radical scavenging assay

The nitric oxide scavenging activity of extracts on nitric oxide radical was measured according to the prescribed method [55]. Sodium nitroprusside (5 mM, 1 ml) in PBS (0.1 M, 7.4 pH) was mixed with 3 ml of different conc. of the extract and incubated at 25°C for 150 minutes. 0.5 ml of the samples was mixed with 0.5 ml of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% napthylethylenediamine dihydrochloride) and absorbance was measured at 546 nm.

Reducing power assay

The reducing power of extracts was evaluated according to the method described by Yen and Chen [53] with slight modification. Briefly, different amount of extracts (100 –700µg/ml) were incubated with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide (K_3Fe [CN] 6) at 50°C for 20 minutes. The reaction was terminated by adding 2.5 ml of 10% TCA solution and the mixture was centrifuged at 3000 rpm for 10 minutes. The supernatant (1.0 ml) was mixed with 2.5 ml of distilled water and 1.0 ml of 0.1% ferric chloride ($FeCl_3$) solution and absorbance was measured at 700 nm after incubation at room temperature for 10 minutes. Quercetin and Butylated hydroxy toluene (5-50 µg/ml) were used as positive control and experiments were performed in triplicate.

Ferric reducing activity

The ferric reducing antioxidant power (FRAP) assay was conducted according to standard protocol [56]. The method is based on the reduction of a ferric 2, 4, 6-tripyridyl-s-triazine complex (Fe3+-TPTZ) by antioxidants to the ferrous form (Fe2+-TPTZ). FRAP reagent was prepared freshly by mixing 2.5 mL of TPTZ solution (10 mM in 40 mM HCl) and FeCl3 (20 mM) in 25 mL of acetate buffer (300 mM and pH 3.6). The light blue Fe3+-TPTZ reagent changes to dark blue after contact with an antioxidant, due to the formation of Fe2+-TPTZ.

Absorbance was monitored at 593 nm for two different concentrations (100 and 200 µg/mL) of extracts in FRAP reagent. All the results were based on three separate experiments and antioxidant capacity was expressed as µM $FeSO_4$/mg of dry extract. Quercetin and butylated hydroxy toluene were used as positive control.

Total antioxidant capacity

0.4 ml of extract (1 mg/ml) dissolved in water was combined in test tube with 4 ml of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated at 95°C for 90 minutes. After cooling to room temperature, the absorbance was measured at 695 nm. The antioxidant activity was expressed as the number of equivalents of ascorbic acid [57,58].

Statistical analysis

All assays were carried out in triplicates and results were analyzed statistically using one-way Analysis of Variance and expressed as mean ± SD. Statistical analysis was performed using GraphPad (GraphPad prism 5.00 for Windows, San Diego California USA).

RESULTS AND DISCUSSION

Preliminary phytochemical screening

The results of preliminary qualitative phytochemical screening of all three *Cassia* species are shown in Table 1. The screening test demonstrated the presence of alkaloids, flavonoids, reducing sugar, saponins, steroids and tannins in all three species. Cardiac glycosides reported in *C. occidentalis* and *C. uniflora*, while terpenoids in *C. tora*. These active phytochemicals play a vital role in the treatment of different ailments, such as tannins possess anti-inflammatory and anticancer activity [59,60]; flavonoids have reported for its antioxidant, anti-inflammatory and anticancer property [61], alkaloids possess antileukemic, anticancer, antimicrobial and cytotoxic activities [62,63], saponin are antimicrobial agent and maintain the blood cholesterol level [64] and terpenoids have been reported to have anti-inflammatory, antioxidant, and neuroprotective activities [65].

Total phenolic content

Polyphenols are the major plant compounds with antioxidant activity, is believed to be mainly due to their redox properties [66] that play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, as well as decomposing peroxides. The results of the total phenolic content of the leaf extracts of three *Cassia* sp. are presented in Table 2. The quantity of total phenolics varied widely among the different analyzed extracts and ranged from 146 to 276 µg/mg of extract. Such variation could be due to the presence of other constituents and/or the presence of different types of phenols in the plant extracts. The leaf extracts of *C. tora* contained significantly lower amount (146.75±0.46 µg gallic acid equivalents (GAE)/mg dry weight) of total phenolics than those of *C. occidentalis* and *C. uniflora*. The highest total phenolic content (276.54±0.31 µg GAE/mg dry weight) was found in the *C. uniflora* followed by *C. occidentalis*. Arya and Yadav [67,68] have reported 13.15 µg/mg phenolic content in dry methanolic leaves extract of *C. tora* and *C. occidentalis* with 21.3 µg/mg content in dry methanolic leaves extract, these values are lower than those found in the present study. Previous research studies have shown that the amount of polyphenolics in plants depend on biological factors (genotype, organ, and ontogeny), edaphic factors and environmental (temperature, salinity, water stress, and light intensity) conditions. Also, the solubility of phenolic compounds is governed by the type of solvent (polarity) used, the degree of polymerization of phenolics and their interaction [69,70].

Total flavonoid contents

Flavonoids are the most common and widely distributed group of plant phenolic compounds, which are usually attributed to their antioxidant properties [71]. In the present study, the total flavonoid content of all three *Cassia* species was also varied and reported in Table 2. *C. uniflora* revealed the highest flavonoid content (43.71±0.082 µg RE/mg dried extract) followed by *C. tora* (34.19±0.043 µg RE/mg dried extract) and *C. occidentalis* (11.24±0.043 µg RE/mg dried extract). It is well known that both genetic and environmental factors play important roles on flavonoid composition of plants [72]. Therefore, these factors would be the key point for affecting the flavonoid content of all three *Cassia* species.

FTIR analysis

Published research literature reveals that many researchers employed the FTIR spectrum as a tool for differentiating, classifying, and discriminating closely related plants and other organisms [51]. The results confirmed the presence of various functional groups such as aldehydes, alcohols, carboxylic acid, alkenes, aromatic compounds, esters, nitro compounds, and alkyl halides are in all the three species

Table 1: Preliminary phytochemical screening of *Cassia* species

Phytochemical constituents	*C. occidentalis*	*C. tora*	*C. uniflora*
Alkaloid	+	+	+
Cardenoloid	+	-	+
Caumarin	-	-	-
Flavonoid	+	+	+
Reducing sugar	+	+	+
Sapponin	+	+	+
Steroid	+	+	+
Tannin	+	+	+
Terpenoid	-	+	-

Table 2: Total phenol and flavonoid content of leaves of three *Cassia* species

Sample	Mean±SD (µg/mg)	
	Phenols	Flavonoids
Cassia ocidentalis	173.45±0.067	11.24±0.043
Cassia tora	146.75±0.046	34.19±0.043
Cassia uniflora	276.54±0.031	43.71±0.086

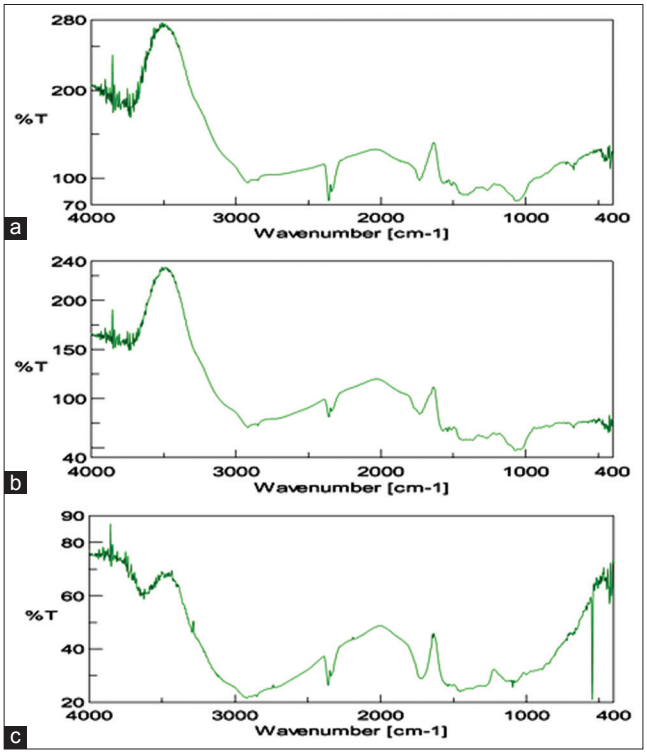

Fig. 1: Fourier transform infrared spectroscopic spectrum of *Cassia* species (a) *Cassia occidentalis*, (b) *Cassia tora*, (c) *Cassia uniflora*

Table 3: FTIR spectral peak values and functional groups obtained for the leaf of three *Cassia* species

Functional groups	Wave number (1/cm)					
	C. tora	Visible intensity	*C. occidentalis*	Visible intensity	*C. uniflora*	Visible intensity
Carboxylic acid						
O-H bend	917.95	m	-	-	-	-
O-H stretch	-	-	2918.73	m	2917.77	m
Esters						
C=O stretch	1717.3	s	1070.3	m	1715.37	s
Aldehydes						
C=O stretch	1732.73	s	1732.73	s	1731.76	S
H–C=O: C–H stretch	-	-	-	-	2725.79	m
Nitro compounds						
N-O symmetric and asymmetric stretch	1539.88	s	1509.03	s	1538.92	s
N–O symmetric stretch	-	-	1339.32	m	-	-
Aromatic compounds						
C–C stretch (in–ring)	1435.74	m	1419.35	m	1455.03	m
Alkanes						
C–H rock	1363.43	m	-	-	-	-
C–H bend	-	-	1455.99	m	1455.03	m
Aromatic amines						
C-N stretch	1270.86	s	-	-	1296.89	s
Alkyl halide						
C–H wag (–CH_2X)	1270.86	m	1265.07	m	-	-
C–Br stretch	-	-	-	-	547.68	m
Ethers						
C–O stretch	1071.26	s	-	-	1296.89	s
Aliphatic amines						
C–N stretch	1071.26	m	-	-	1093.44	m
Alkenes						
=C–H bend	917.95	s	916.98	s	2917.77	s
Amine						
N–H wag	894.80	s, b	-	-	889.98	s, b
Alkynes						
–C≡C–H: C–H bend	668.21	b, s	-	-	-	-
–C≡C– stretch	-	-	-	-	2184.95	w
Carbonyls						
C=O stretch	-	-	1732.73	s	1715.37	s
Phenols						
O–H stretch, H–bonded	-	-	3212.83	s, b	-	-
Ketones						
C=O stretch	-	-	-	-	1715.37	s

C. occidentalis: Cassia occidentalis, C. tora: Cassia tora, C. uniflora: Cassia uniflora, FTIS= Fourier transform infrared spectroscopy, m= medium; w= weak, s= strong, n= narrow, b= broad, sh= sharp

studied (Fig. 1 and Table 3). Moreover, aromatic amines, ethers are observed in *C. occidentalis* and *C. uniflora* and carbonyl in *C. tora* and *C. uniflora* while phenol reported exclusively in *C. tora*.

In vitro free radical quenching

Antioxidant screening of three *Cassia* species was determined using different *in vitro* analytical assay. Since, antioxidant activity cannot be evaluated using a single test [73]. All antioxidant assays provide considerable support to antioxidant prospective of the plant in comparison with standard ascorbic acid.

DPPH assay

The DPPH has been extensively used as a free radical to evaluate reducing substances [70,74]. The results of DPPH free radical scavenging activity of methanolic extract of the three *Cassia* species and the positive control ascorbic acid are shown in Fig. 2 and Table 4. In DPPH assay, all extracts showed a notable radical scavenging activity in a dose-dependent manner within a certain range and were significantly different ($p<0.05$). Among them, *C. occidentalis* showed the highest DPPH scavenging activity with the lowest IC_{50} value of 58.55±0.213 µg/ml, followed by *C. tora* (61.55±0.318 µg/mL) and *C. uniflora* (105.63±0.479 µg/mL). Previous studies reported that ethanolic and aqueous extracts of *C. occidentalis* exhibited modest antioxidant potential ($IC_{50}37_{\mu g}$/ml) compared to the present study in aqueous methanol extract [75,76]. However, methanol extract of *C. tora* showed lower antioxidant activity [77-79].

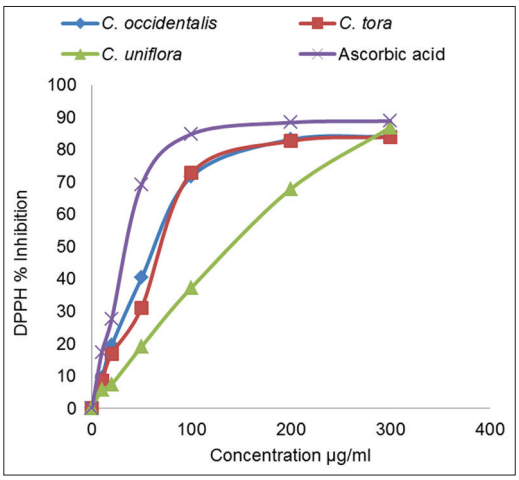

Fig. 2: 2, 2 diphenyl-1-picrylhydrazyl radical scavenging

ABTS assay

The ABTS assay is also commonly used to study antioxidant capacity in plants based on the capacity of the extracts to scavenge the radical cation $ABTS^{\bullet+}$ generated during the application of this method [80-82]. All the three extracts of *Cassia* species scavenged ABTS radical in a

concentration dependent manner and showed significant difference at p<0.05. The highest activity was obtained from the *C. uniflora* extract with a IC_{50}value of 0.074±0.003 µg/ml equivalent to ascorbic acid (Table 4), followed by *C. occidentalis* and *C. tora* with IC_{50} values of 24.388±0.183 and 29.388±0.183 µg/ml equivalent to ascorbic acid, respectively (Fig. 3).

Nitric oxide assay

All the extracts caused considerable dose-dependent nitric oxide scavenging in comparison to the standard ascorbic acid. This may be due to the antioxidants present in the extract. The percentages of inhibition were increased with increasing concentration of the extracts (Fig. 4). The IC_{50} values (Table 4) for *C. occidentalis* was 1.584±0.095 µg/ml, followed by *C. tora* and *C. uniflora* with 7.159±0.053 and 8.23±0.034 µg/ml, respectively. The present findings of nitric oxide scavenging activity of *C. occidentalis* and *C. tora* are in agreement with those in previously published literature [67,68,77,78].

Reducing power assay

The reducing power of *C. occidentalis, C. tora* and *C. uniflora* extracts are shown in Fig. 3. In the reducing power assay, a stronger absorbance indicates a higher reducing power. Extracts of all three species of *Cassia* were able to catalyze reduction of Fe (III). *C. occidentalis* had much higher reducing potential than *C. tora* and *C. uniflora* (Fig. 5). The methanolic extract of *C. uniflora* exhibited the lowest reductive power values. The reducing power assay is often used to evaluate the ability of natural antioxidant to donate an electron or hydrogen [83], which is an important mechanism of phenolic antioxidant action [84].

FRAP assay

The FRAP method is commonly employed to determine the antioxidant activity of plant materials by measuring the capacity of the extracts to reduce ferric complexes to the ferrous form [82,85]. The FRAP values of three aqueous methanolic leaves of *Cassia* species analyzed ranged from 76.69±0.224 to 262.67±0.163 µg/mg DW (Table 5). The highest FRAP value was obtained in *C. uniflora*, while the lowest was in *C. tora*. The extracts of *C. tora* and *C. uniflora* expressed electron donating activity, but their power was inferior to ascorbic acid, which is known to be a strong reducing agent.

Total antioxidant assay

Total antioxidant capacity by ammonium molybdenum method assay is based on the reduction of Mo (VI) to Mo (V) by the antioxidant sample. This method has been routinely used to evaluate the antioxidant capacity of extracts [57]. Total antioxidant capacity of the studied *Cassia* species was expressed as the number of equivalents of ascorbic acid (Table 5). Higher activity (48.82±0.14 µg/mg of dry extract) was observed for *C. tora* followed by *C. occidentalis* (36.62±0.19 µg/mg of dry extract) and *C. uniflora* showed the lowest activity (20.52±0.40 µg/mg).

Table 4: IC_{50} values of *Cassia* species on tested radicals

Plants	IC_{50} value (µg/ml)		
	DPPH	ABTS	Nitric oxide
C. occidentalis	58.55±0.213	24.388±0.183	1.584±0.095
C. tora	61.55±0.318	29.388±0.368	7.159±0.053
C. uniflora	105.63±0.479	0.074±0.003	8.23±0.034

Data presented as mean±SD each triplicate test. p<0.05 was considered significant

Table 5: Total antioxidant activity of three *Cassia* species

Sample	Total antioxidant activity (µg/mg)	
	FRAP	Ammonium molybdate
Cassia occidentalis	76.69±0.224	36.62±0.19
Cassia tora	82.16±0.103	48.82±0.14
Cassia uniflora	262.67±0.163	20.52±0.40

Data are presented as mean±SD each triplicate test. p<0.05 was considered significant

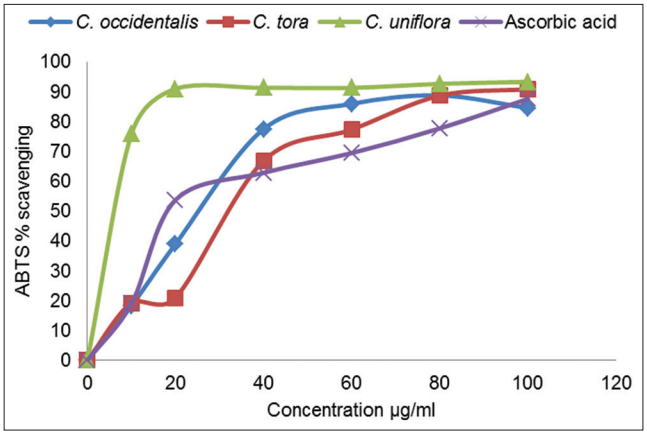

Fig. 3: 2,2-azinobis [3,ethyl-benzothiazoline-6s-sulphonic acid] radical scavenging

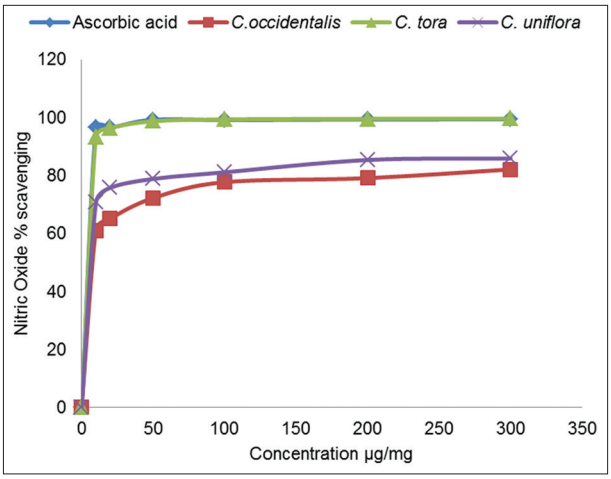

Fig. 4: Nitric oxide scavenging

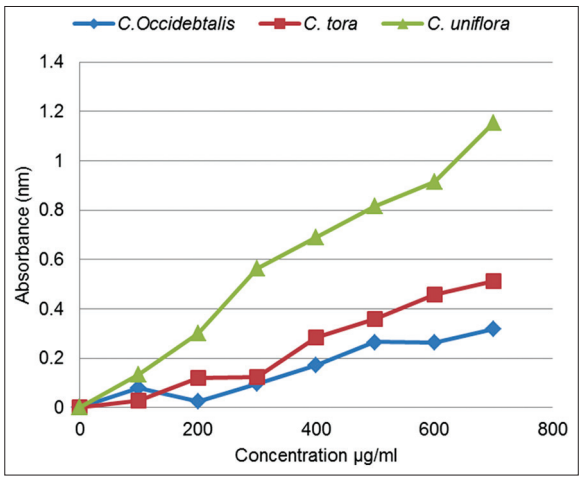

Fig. 5: Reducing power assay

CONCLUSION

The present study deals with the comparative antioxidant and phytochemical screening of three *Cassia* species. Based on the results,

it revealed that all the species of *Cassia* had rich in phenolic and flavonoid constituents (with increasing order *C. occidentalis*, C. *tora* and *C. uniflora*). FTIR results revealed the presence of various functional groups such as aldehydes, alcohols, carboxylic acid, alkenes, aromatic compounds, esters, nitro compounds, and alkyl halides are in all the three species. Overall, all species exhibited the dose-dependent antioxidant potential; it is *Cassia occidentalis* that showed the highest antioxidant activity. Further studies are needed pertaining to *in vivo* screening and identification of compounds responsible for the antioxidant activities that leads to potent source of natural antioxidants.

ACKNOWLEDGMENTS

The authors are grateful to Principal, S. N. Arts, D.J.M. Commerce and B.N.S. Science College, Sangamner for providing necessary facilities.

REFERENCES

1. El-Hashash MM, Abdel-Gawad MM, El-Sayed MM, Sabry WA, Abdel-Hameed el-SS, Abdel-Lateef Eel-S. Antioxidant properties of methanolic extracts of the leaves of seven Egyptian *Cassia* species. Acta Pharm 2010;60:361-7.
2. Anonymous. The Wealth of India. Raw Materials. Vol. II. Delhi: CSIR; 1950.
3. Ganapaty S, Thomas PS, Ramana KV, Vidyadhar K, Chakradhar VA. Review of phytochemical studies of *Cassia* species. J Nat Remedies 2002;2:102-20.
4. Liogier HA. Descriptive Flora of Puerto Rico and Adjacent Islands. Spermatophyla. Vol. 2. Rio Piedras, PR: Editorial de la Universidad de Puerto Rico; 1988. p. 481.
5. Stevens WD, Ulloa UC, Pool A, Monitel OH. Flora de Nicaragua. Monographs of Systematic Botany, 85. St. Louis, MO: Missouri Botanical Garden Press; 2001.
6. Khare CP. Indian Medicinal Plants: An Illustrated Dictionary. US: Springer; 2007.
7. Kirtikar KR, Basu BD. Indian Medicinal Plant. Allahabad: Allahabad Lalit Mohan Basu; 1933.
8. Nadkarni AK. Indian Materia Medica. Bombay: Popular Publication; 1976.
9. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. New Delhi: CSIR; 1980.
10. Warrier PK, Nambiar VP. Indian Medicinal Plants: A Compendium of 500 Species. Hyderabad: Orient Orient Longman Ltd.; 1994.
11. Patil MV, Patil DA. Ethnobotany of Nasik District of Maharashtra. New Delhi: Daya Books; 2006.
12. Pardo de Tavera TH. The Medicinal Plants of the Philippines. Philadelphia: P. Blakiston's Son & Co.; 2008.
13. Duke JA. Duke's Handbook of Medicinal Plants of Latin America. Boca Raton: CRC Press Taylor & Francis Group; 2009.
14. Coimbra R. Manual de Fitoterapia. Belém, Brazil: Editora CEJUP; 1994.
15. Di Stasi LC, Hiruma - Rasp AC. Plants Medicine on Amazônia and on Forest Atlantic. Publishing company UNESP São Paul; 2002 p.604.
16. Silva MG, Aragão TP, Vasconcelos CF, Ferreira PA, Andrade BA, Costa IM, *et al*. Acute and subacute toxicity of *Cassia occidentalis* L. stem and leaf in Wistar rats. J Ethnopharmacol 20112;136(2):341-6.
17. Daniel M. Medicinal Plants: Chemistry and Properties. Hauppauge, NY, USA: Scientific Publishers; 2005. p. 175.
18. Rai PM, Shok M. Anthraquinone glycosides from plant parts of *Cassia occidentalis*. Indian J Pharm Sci 1983;45:87-8.
19. Tiwari RD, Singh J. Anthraquinone pigments from cassia occidentalis. Planta Med 1977;32(4):375-7.
20. Tiwar RD, Singh J. Flavonoids from the leaves of *Cassia occidentalis*. Phytochemistry 1977;16:1107-8.
21. Hussain HS, Deeni YY. Plants in Kano ethnomedicine: Screening for antimicrobial activity and alkaloids. Int J Pharmacogn 1991;29:51-6.
22. Muanza DN, Dangala NL, Mpay O. Zairean medicinal plants as diarrhea remedies and their antibacterial activities. Afr Study Monogr 1993;14:53-63.
23. Ali MS, Azhar I, Amtul Z, Ahmad VU, Usmanghani K. Antimicrobial screening of some *Caesalpiniaceae*. Fitoterapia 1999;70:299-304.
24. Tona L, Ngimbi NP, Tsakala M, Mesia K, Cimanga K, Apers S, *et al.* Antimalarial activity of 20 crude extracts from nine African medicinal plants used in Kinshasa, Congo. J Ethnopharmacol 1999;68(1-3):193-203.
25. Tona L, Cimanga RK, Mesia K, Musuamba CT, De Bruyne T, Apers S, *et al. In vitro* antiplasmodial activity of extracts and fractions from seven medicinal plants used in the democratic republic of Congo. J Ethnopharmacol 2004;93(1):27-32.
26. Sadique J, Chandra T, Thenmozhi V, Elango V. Biochemical modes of action of *Cassia occidentalis* and *Cardiospermum halicacabum* in inflammation. J Ethnopharmacol 1987;19(2):201-12.
27. Arya V, Yadav JP. Antioxidant properties of the methanol extracts of the leaves seeds and stem of *Cassia occidentalis*. Res J Med Plants 2011;5:1-10.
28. Sheeba Rani M, Emmanuel S, Sreekanth MR, Ignacimuthu S. Evaluation of *in vivo* antioxidant and hepatoprotective activity of *Cassia occidentalis* L. against paracetamol induced liver toxicity in rats. Int J Pharm Pharm Sci 2010;2:67-70.
29. Jain S, Patil UK. Phytochemical and pharmacological profile of *Cassia tora* L. - An overview. Indian J Nat Prod Resour 2010;1:430-7.
30. Rejiya CS, Cibin TR, Abraham A. Leaves of *Cassia tora* as a novel cancer therapeutic – An *in vitro* study. Toxicol *In Vitro* 2009;23(6):1034-8.
31. Daiziel AJ. The Useful Plants of West Tropical Africa. London: Crown Agents for Overseas Governments and Administrations; 1995.
32. Irvine FR. Woody Plants of Ghana. London: Oxford University Press; 1961.
33. Chidume FC, Kwanashie HO, Adekeye JO, Wambebe C, Gamaniel KS. Antinociceptive and smooth muscle contracting activities of the methanolic extract of *Cassia tora* leaf. J Ethnopharmacol 2002;81(2):205-9.
34. WHO. Medicinal Plants of Vietnam. Manila: World Health Organization; 1989.
35. Maity TK, Mandal SC, Mukharjee PK, Saha K, Das J, Pal M, *et al*. Studies on Anti-inflammatory effect of *Cassia tora* leaf extract (Fam.- Leguminosae). Phytother Res 1998;12:221-3.
36. Maity TK, Mandal SC, Mukharjee PK, Saha K, Das J, Pal M, *et al*. Evaluation of hepatoprotective potential of *Cassia tora* leaf extract. Nat Prod Sci 1997;3(2):122-6.
37. Dhanasekaran M, Ignacimuthu S, Agastian P. Potential hepatoprotective activity of ononitol monohydrate isolated from *Cassia tora* L. on carbon tetrachloride induced hepatotoxicity in wistar rats. Phytomedicine 2009;16(9):891-5.
38. Chidume FC, Kwanashie HO, Adekeye JO, Wambebe C, Gamaniel KS. Antinociceptive and smooth muscle contracting activities of the methanolic extract of *Cassia tora* leaf. J Ethnopharmacol 2002;81(2):205-9.
39. John J, Mehta A, Mehta P. Evaluation of antioxidant and anticancer potential of *Cassia tora* leaves. Asian J Tradit Med 2012;7:260-7.
40. Mukherjee PK. Quality Control of Herbal Drug. New Delhi: Business Horizons; 2002.
41. Amala S. Traditional Medicines for Modern Times. USA: Taylor and Francis; 2005.
42. Yun-Choi HS, Kim JH, Takido M. Potential inhibitors of platelet aggregation from plant sources, V. Anthraquinones from seeds of *Cassia obtusifolia* and related compounds. J Nat Prod 1990;53(3):630-3.
43. Shibata S, Morishita E, Kaneda M, Kimura Y, Takido M, Takahashi S. Chemical studies on the oriental plant drugs. XX. The constituents of *Cassia tora* L 1. The structure of torachrysone. Chem Pharm Bull (Tokyo) 1969;17(3):454-7.
44. Cooke T. The Flora of the Presidency of Bombay. Calcutta: BSI, 1955.
45. Pradhan SG, Singh NP. Flora of Ahmednagar District (M.S). Dehradun, India: Bishen Singh Mahendra Pal Singh; 1999.
46. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed. London: Academic Press; 1998.
47. Mahida Y, Mohan JS. Screening of Indian plant extracts for antibacterial activity. Pharm Biol 2006;44(8):627-31.
48. Trease GE, Evans WC. Pharmacognosy. 12th ed. London: Baillieere Tindal; 1983.
49. Zou Y, Lu Y, Wei D. Antioxidant activity of a flavonoid-rich extract of Hypericum perforatum L. *in vitro*. J Agric Food Chem 2004;52(16):5032-9.
50. Cliffe S, Fawer M, Maier G, Takata K, Ritter G. Enzyme assays for the phenolic content of natural juices. J Agri Food Chem 1994;42:1824-8.
51. Janakiraman N, Sahaya SS, Johnson M. UV-VIS and FTIR spectroscopic studies on *Peristrophe bicalyculata* (Retz.) Nees. Asian J Pharm Clin Res 2011;4(4):125-9.
52. Basavegowda N, Idhayadhulla A, Lee YR. Phyto-synthesis of gold nanoparticles using fruit extract of *Hovenia dulcis* and their biological activities. Ind Crops Prod. 2014;52:745-51.
53. Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their anti-mutagenicity. J Agric Food Chem 1995;43:27-32.
54. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M,

Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 1999;26(9-10):1231-7.
55. Ilavarasan R, Mallika M, Venkataraman S. Anti-inflammatory and antioxidant activites of *Cassia fistula Linn* bark extract. Afr J Tradit 2005;(1):70-85.
56. Benzie IF, Strain JJ. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol 1999;299:15-27.
57. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem 1999;269(2):337-41.
58. Shirwaikar A, Rajendran K, Kumar CD. *In vitro* antioxidant studies of *Annona squamosa* Linn. leaves. Indian J Exp Biol 2004;42(8):803-7.
59. Olajide OA, Aderogba MA, Adedapo AD, Makinde JM. Effects of *Anacardium occidentale* stem bark extract on *in vivo* inflammatory models. J Ethnopharmacol 2004;95(2-3):139-42.
60. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis 1989;10(6):1003-8.
61. Ogunleye DS, Ibitoye SF. Studies of antimicrobial activity and chemical constituents of *Ximenia americana*. Trop J Pharm Res 2003;2(2):239-41.
62. Decorti RC, Creacy WA. In: Dekker M, Taylor W, Fanworths NR. The Cantharadus Alkaloids. New York: Taylor W and Fanworths NR Publishers; 1975. p. 237-78.
63. Oloyede KG, Oke MJ, Raji Y, Olugbade AT. Antioxidant and anticonvulsant alkaloids in *Crinum ornatum* bulb extract. World J Chem 2010;5:26-31.
64. Cheeke PP. Surprising benefits of desert plants. Corvallis, Oregon: Oregon University, in the Linus Paulin Institute; 2005.
65. Mu L, Kou J, Zhu D, Yu B. Comparison of neuroprotective effects of flavonoids, terpenoids, and their combinations from *Ginkgo biloba* on ischemia-reperfusion-injured mice. Pharm Biol 2007;45:728-33.
66. Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. J Agric Food Chem 2001;49(11):5165-70.
67. Arya V, Yadav JP. Antioxidant activity and total phenolics in leaves extracts of *Cassia Tora* L. Pharmacol Online 2010;2:1030-6.
68. Arya V, Yadav JP. Comparative assessment of relative antioxidant activity of sequential leaf extracts of *Cassia occidentalis* L. and *C. Tora* L. Pharmacol Online 2011;1:529-43.
69. Ksouri R, Megdiche W, Falleh H, Trabelsi N, Boulaaba M, Smaoui A, *et al.* Influence of biological, environmental and technical factors on phenolic content and antioxidant activities of Tunisian halophytes. C R Biol 2008;331(11):865-73.
70. Khoudja NK, Makhlouf LB, Madani K. Antioxidant capacity of crude extracts and their solvent fractions of selected *Algerian lamiaceae*. Ind Crops Prod 2014;52:177-82.
71. Ismail HI, Wei CK, Mariod AA, Ismail M. Phenolic content and antioxidant activity of cantaloupe (*Cucumis melo*) methanolic extracts. Food Chem 2010;119:643-7.
72. Wang SS, Wang DM, Pu WJ, Li DW. Phytochemical profiles, antioxidant and antimicrobial activities of three *Potentilla* species. BMC Complement Altern Med 2013;13:321.
73. Apak R, Güçlü K, Demirata B, Ozyürek M, Celik SE, Bektasoglu B, *et al.* Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. Molecules 2007;12(7):1496-547.
74. Cotelle N, Bernier JL, Catteau JP, Pommery J, Wallet JC, Gaydou EM. Antioxidant properties of hydroxy-flavones. Free Radic Biol Med 1996;20(1):35-43.
75. Sathya AV, Ambikapathy V, Panneer Selvam A. Studies on the phytochemistry, antimicrobial activity and antioxidant properties of *Cassia occidentalis* L. Asian J Plant Sci Res 2012;2(4):530-3.
76. Gbadegesin MA, Odunola OA. *In vitro* antioxidant/radical scavenging activities and hepatoprotective roles of ethanolic extract of *Cassia occidentalis* leaves in sodium arsenite-treated male wistar rats. Br J Med Med Res 2013;3:2141-56.
77. Sirappuselvi S, Chitra M. In *vitro* antioxidant activity of *Cassia tora* Lin. Int Res J Biol Sci 2012;1:57-61.
78. John J, Mehta A, Mehta P. Evaluation of antioxidant and anticancer potential of *Cassia tora* leaves. Asian J Tradit Med 2012;7(6):260-7.
79. Sahu RK, Kar M, Routray R. DPPH Free radical scavenging activity of some leafy vegetables used by tribals of Odisha. India. J Med Plants Stud 2013;1(4):21-7.
80. Kim DO, Lee KW, Lee HJ, Lee CY. Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. J Agric Food Chem 2002;50(13):3713-7.
81. Gouveia S, Gonc alves J, Castilho PC. Characterization of phenolic compounds and antioxidant activity of ethanolic extracts from flowers of *Andryala glandulosa* ssp. varia (Lowe ex DC.) R. Fern., an endemic species of Macaronesia region. Ind Crops Prod 2013;42:573-82.
82. Xi W, Zhang Y, Sun Y, Shen Y, Ye X, Zhou Z. Phenolic composition of Chinese wild mandarin (Citrus reticulate Balnco.) pulps and their antioxidant properties. Ind Crops Prod 2014;52:466-74.
83. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthone on the auto oxidation of soybean in cylcodextrin emulsion. J Agric Food Chem 1992;40:945-8.
84. Dorman HJ, Peltoketo A, Hiltunen R, Tikkanen MJ. Characterisation of the antioxidant properties of deodourisation aqueous extracts from selected *Lamiaceae* Herbs. Food Chem 2003;83:255-6.
85. Contreras-Calderón J, Calderón-Jaimes L, Guerra-Hernández E, García-Villanova B. Antioxidant capacity, phenolic content and vitamin C in pulp, peel and seed from 24 exotic fruits from Colombia. Food Res Int 2011;44:2047-53.