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ORIGINAL ARTICLE

Effects of temperature and solvent on antioxidant properties of curry leaf (*Murraya koenigii* L.)

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Abstract Total polyphenol content (TPC) and antioxidant activities of curry leaf extracts (hexane, chloroform, ethanol, ethanol-water (1:1) and water at ambient (AT, 25 °C) and boiling temperature (BT) (Soxhlet extraction), were determined by DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt), and total reductive potential assays. TPC was in the order ethanol-water (1:1) (AT) > water (AT) > chloroform (AT) > ethanol-water (1:1) (BT) > hexane (AT) > ethanol (BT) > water (BT) > hexane (BT). Ethanol-water (AT) had the maximum TPC of 501± 4.6 mg/g GAE and 82% radical scavenging activity (RSA) at 10 μg/ml level (DPPH) and 100% RSA (ABTS) at 10 μg/ml and at hot conditions (ethanol-water (BT)) had less TPC (28.7±0.9%), and 43% RSA by DPPH and 53.6% by ABTS assays. Hot extracts had lesser antioxidant activities than ambient extracts. The best solvent system for getting maximum antioxidant activity from curry leaves was ethanol-water (1:1)-(AT).

Keywords Curry leaf · *Murraya koenigii* · Antioxidant · DPPH radical scavenging activity · Reductive potential · ABTS radical cation decolorization assay

Introduction

Antioxidants stop unwanted oxidation in the body, which involve the formation of free radicals and further deteriorate the condition of the body (Arulselvan and Subramanian 2007). Reactive oxygen species (ROS), causing damage to DNA, proteins and lipids, have been associated with carcinogenesis, coronary heart disease and many other health problems (Black et al. 1995; Cadenas and Davies 2000). Minimizing oxidative damage may well be one of the most important approaches to prevention of these oxidative stress-related diseases and health problems, since antioxidants terminate direct ROS attacks and radical-mediated oxidative reactions (Burits and Bucar 2000; Yu et al. 2002). The auto oxidation of fats is a major problem due to deterioration in the quality of foods mediated oxidative reactions. Safeguarding fats against oxidation is normally done by restricting the access of oxygen or adding antioxidants. The most commonly added antioxidants are synthetic phenols, such as butylated hydroxy toluene (BHT) and butylated hydroxy anisole (BHA). Their safety however is doubtful. The antioxidation is an extremely significant activity, which can be used as a preventive measure against a number of diseases. Therefore the worldwide research is now focused on natural antioxidants.

Curry leaf is a traditional spice used in South India for all curry preparations. The plant *Murraya koenigii* (L.) Spreng, belonging to the family *Rutaceae* is native to India and distributed in most part of Southern Asia. The leaves increase digestive secretions and relieve nausea, indigestion, and vomiting (Arulselvan and Subramanian 2007). Phytochemical studies on leaves, stem, bark and root of this plant have resulted in the isolation of carbazole alkaloids including murrayanine, girinimbine, mahanimbine, murrayafoline-A and one triterpene (Chakraborty et al. 1978; Ito et al. 1993; Baker et al. 2007). Five more carbazole alkaloids viz, euchrestine B, bismurrayafoline E, mahanine, mahanimbicine and mahanimbine were reported from curry leaves and their radical RSA were ascertained (Tachibana et al.

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2001). This species is known to possess anti-inflammatory, antidysenteric, antioxidant, antidiabetic and has diverse pharmacological properties (Arulselvan and Subramanian 2007). An antioxidant protein was isolated from curry leaves which showed apparent molecular weight of 35 kDa (Ningappa et al. 2008). Two compounds of high activity viz, koenigine and mahanimbine, were reported from the acetone extract of curry leaves (Rao et al. 2007). The enzymatic antioxidant status of peroxidase, polyphenol oxidase and non-enzymatic antioxidants such as ascorbic acid, reducing sugar, phenol and proteins were reported to be more in tender leaves (Mahajan and Patil 2004) compared to mature leaves.

A new dimeric carbazole alkaloid, 8,10′-[3,3′,11,11′ -tetrahydro-9,9′-dihydroxy-3,3′,5,8′-tetramethyl-3, 3′-bis(4-methyl-3-pentenyl)]bipyrano[3,2-a]carbazole, was isolated from the CH_2Cl_2 extract of curry leaf and its antioxidant properties together with other carbazole alkaloids viz, O-methylmurrayamine A, O-methylmahanine, isomahanine, bismahanine and bispyrayafoline, were evaluated on the basis of the oil stability index and RSA against DPPH radical. It is suggested that an aryl hydroxyl substituent on the carbazole ring plays a role in stabilizing the thermal oxidation and rate of reaction against DPPH radical (Tachibana et al. 2003).

The present work was carried out to study the effect of temperature and different solvents on the antioxidant property of curry leaves. The curry leaves were extracted with solvents of varying polarity at room temperature and by Soxhlet extraction method.

Materials and methods

Plant material Curry leaves were procured from Kerala Agricultural University Plantation, Trivandrum, and the voucher specimen was deposited in the herbarium. Two hundred grams of the curry leaves were dried in an air oven (Rivotek, Kochin Inlab Equipments India Pvt Ltd, India) for 3 days at 50 °C. After complete drying, the leaves were ground to a fine powder using a domestic electric mixer grinder (Pananasonic MX-216E, Thiruvananthpuram, India).

Chemicals The compounds 2,2′-azinobis (3-ethylbenzo-thiazoline-6- sulphonic acid) diammonium salt (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH) as free radical, gallic acid, 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), potassium ferricyanide, $FeCl_3.3H_2O$, potassium persulphate, sodium phosphate, and sodium carbonate were supplied from Sigma–Aldrich (St. Louis, MO, USA), Folin–Ciocalteu reagent, trichloro-acetic acid, ethanol (EtOH), hexane and chloroform were of analytical grade and procured from Merck (Darmstadt, Germany). All other reagents and solvents used were of AR grade.

Extraction at ambient temperature Curry leaf powder (1 g) was extracted separately with double distilled water, EtOH and water mixture (1:1), EtOH, hexane and chloroform (50 ml). The suspension was shaken in a shaking water bath for 30 min at room temperature (25 °C). The resultant suspension was centrifuged at 10,000 × g for 10 min at 4 °C and filtered through Whatman No. I filter paper, followed by 0.045 μm microbial filter (Sartorius minisart, Hannover, Germany). The extracts after the removal of solvents were lyophilised. Ten mg of each dried extract were dissolved in 0.1 ml of the respective extracting solvent or solvent mixture and made up to 10 ml with distilled water. The solution was filtered using a 0.45 μm microbial filter and stored at −20 °C for further studies.

Soxhlet extraction Curry leaf powder (10 g) was extracted separately in a Soxhlet apparatus (Buchi Extraction System B811, BÜCHI Labortechnik AG, Flawil, Switzerland) with 250 ml of hexane, chloroform, EtOH, EtOH and water mixture (1:1) and distilled water. The extracts after the removal of solvents were stored at 4 °C until used for antioxidant assays. The efficacy of the extracts was quantified based on the dry weight of the whole extract per volume of assay solution.

Total phenolic content (TPC) TPC of extracts was assessed by the Folin–Ciocalteu phenol reagent with gallic acid as standard (Jagadish Singh et al. 2009). The TPC was expressed as gallic acid equivalents (GAE) in mg/g of sample.

DPPH spectrophotometric assay DPPH scavenging activity in the extracts was assessed as described by Burits and

Table 1 Extraction yield and total phenolic content of extracts

Extract	Yield,%	TPC content (GAE equiv), mg/g
Hexane AT	4.8±0.07	244.0±2.5
Hexane BT	12.5±2.10	157.0±1.8
Chloroform AT	6.5±0.08	304.0±0.8
Chloroform BT	9.7±1.11	140.0±3.4
EtOH AT	15.0±1.50	155.0±2.1
EtOH BT	20.6±1.10	178.0±3.2
EtOH-water AT	20.1±0.44	501.4±4.6
EtOH-water BT	22.8±0.80	287.0±0.9
Water AT	17.7±0.62	326.0±1.7
Water BT	20.6±0.80	169.0±3.2

AT Ambient temperature; *BT* Boiling temperature

TPC Total phenolic content; *GAE* Gallic acid equivalent

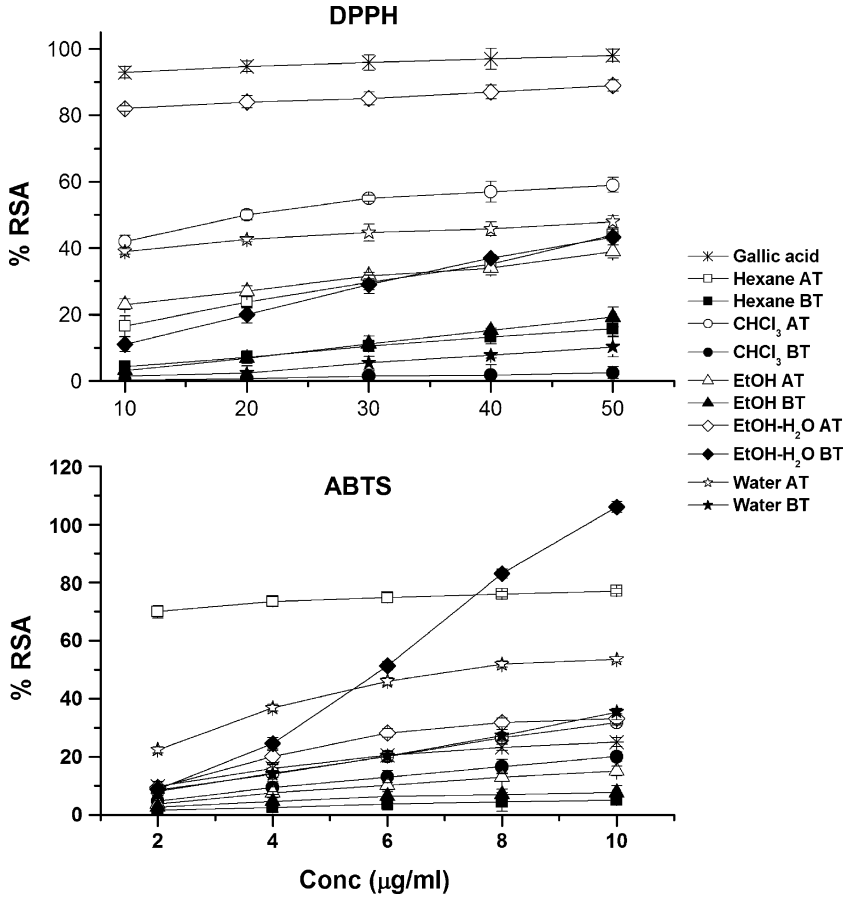

Fig. 1 DPPH and ABTS radical scavenging activities (RSA) of extracts

Bucar (2000). Each extract stock solution (1.0 mg/ml) was diluted to final concentrations ranging from (10–50 μg/ml) in EtOH. One ml of a 0.3 mM DPPH solution was added to 2.5 ml of extract solution of different concentrations and allowed to react at room temperature in dark condition. After 30 min, the absorbance was measured at 518 nm and the percentage scavenging capacity was calculated.

ABTS radical cation decolorization assay The experiments were carried out using an improved ABTS decolorisation assay (Re et al. 1999), which involved the generation of $ABTS^{\bullet+}$ chromophore by the oxidation of ABTS with potassium persulphate. It is applicable for both hydrophilic and lipophilic compounds. The $ABTS^{\bullet+}$ solution was diluted to an absorbance of 0.7±0.05 at 734 nm. Appro-

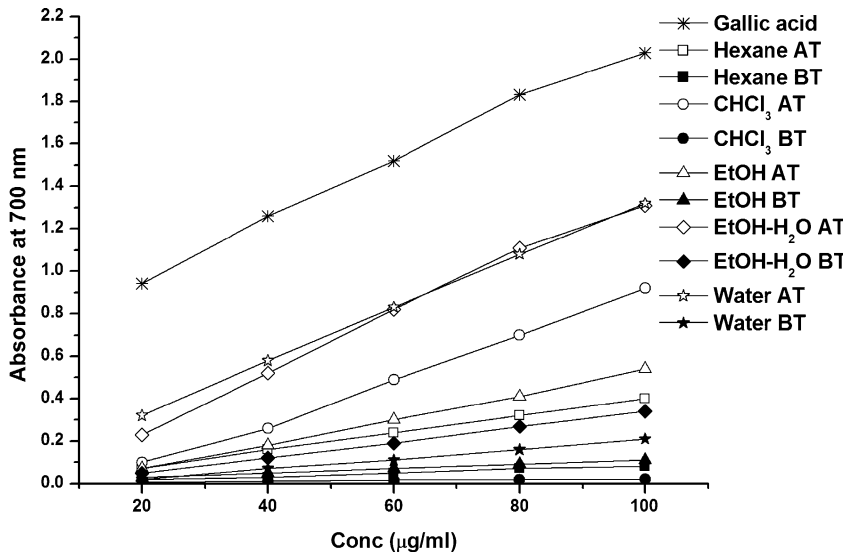

Fig. 2 Reducing power of extracts

priate volumes were taken from stock solution of curry leaves (10 μg/ml) for measurements (total volume of extract solution 1 ml). Absorbance was measured at 7 min after the initial mixing of different concentrations of the extracts with 1 ml of $ABTS^{\bullet+}$ solution. Gallic acid was used as standard.

Reductive potential One ml of the extract (10–100 μg/ml) was mixed with 2.5 ml of 200 mM, pH 6.6 phosphate buffer and 2.5 ml 1% (w/v) potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. Trichloroacetic acid (2.5 ml of 10%) was added to the mixture, which was then centrifuged for 10 min at 1,000 × g; 2.5 ml of the supernatant was diluted with 2.5 ml of distilled water and 0.5 ml 0.1% $FeCl_3$ and the absorbance was read at 700 nm.

Statistical analysis Experimental results were mean ± SD of 3 parallel measurements. The data were analysed by analysis of variance ($p<0.05$) and the means separated by Duncan's multiple range test. Results were processed by Origin Pro 8 and Microsoft Excel 2007 softwares.

Results and discussion

Yield and phenolic content in different extracts Maximum yield was obtained by Soxhlet extraction with EtOH and water mixture (22.8±0.80%) and lowest yield was with hexane at room temperature (4.8±0.07%) (Table 1).

The phenolic content was found to be maximum (501.4 ±4.6 mg/g GAE) in EtOH—water mixture extract (AT) and lowest in chloroform extract (BT) (140±3.4 mg/g GAE) (Table 1). The EtOH extract (BT) has TPC of 287± 0.9 mg/g GAE showing that the temperature used for extraction is important for the maximum content of phenolic compounds and activity. Earlier report showed that the curry leaves had a TPC of 168 mg/g GAE in EtOH-water extract (Ningappa and Srinivas 2008).

DPPH assay Aqueous ethanol (1:1) extract (AT) had highest scavenging activity compared to other extracts (Fig. 1). This is in accordance with earlier finding (Ningappa and Srinivas 2008). The TPC was maximum in aqueous alcohol extract (AT) and had scavenging activities of 82% at 10 μg/ml while control gallic acid had 92% at the same level. The aqueous alcohol extracted at AT was the best system for maximum activity. The extracting temperature had a negative effect on DPPH scavenging activity. Earlier study reported that the DPPH scavenging activity of aqueous alcohol extract as 92% at 20 μg/ml concentration (Ningappa and Srinivas 2008) while the present extract gave 82% at 10 μg/ml. The comparatively higher value of DPPH activity of the present extract may be due to the high content of phenolic compounds.

ABTS assay The ABTS assay is applicable to both lipophillic and hydrophilic compounds. The reaction is pH-independent and a decrease in the $ABTS^{\bullet+}$ concentration is linearly dependent on the antioxidant concentration, including Trolox as a calibrating standard. The results are in accordance with the TPC of extracts. The aqueous EtOH (1:1) extract had maximum activity and minimum was in the hexane (AT) extract (Fig. 1). The aqueous EtOH (1:1) extract (AT) was better than control gallic acid even at 7 μg/ml. The Soxhlet extracts showed lesser antioxidant activity. The activity was reduced by 50% in case of aqueous EtOH extracts (1:1) at AT and BT. The results clearly show the superior capacity of aqueous alcohol (1:1) extract (AT) of curry leaf as measured by ABTS method. No earlier reports are available on the ABTS radical scavenging assays of different extracts.

Total reductive potential The aqueous EtOH (1:1) extract (AT) had maximum reductive potential as measured by ferric ion reduction and minimum was in hexane extract (BT) (Fig. 2). When extracted at BT extracts had lesser reducing power. The lowest activity was shown by chloroform extracts. Earlier work showed that the reductive potential increased with increase in concentration of extracts (Huda et al. 2009).

Conclusion

Ethanol-water (1:1) is the best solvent system for curry leaves both at AT and BT (Soxhlet extraction) for antioxidant activities as assessed by DPPH, ABTS and total reducing power.

References

Arulselvan P, Subramanian SP (2007) Beneficial effects of *Murraya koenigii* leaves on antioxidant defense system and ultrastructural changes of pancreatic β-cells in experimental diabetes in rats. Chem Bio Interact 165:155–164

Baker NHA, Sukari MA, Rahmani M, Shariff AM, Khalid K, Yusuf UM (2007) Chemical constituents from stem barks and roots of *Murraya koenigii* (Rutaceae). Malaysian J Anal Sci 11:173–176

Black HS, Thornby JI, Wolf JE, Goldberg LH, Herd JA, Rosen T, Bruce S, Tschen JA, Scott LW, Jaax S, Foreyt JP, Reusser B (1995) Evidence that a low-fat diet reduces the occurrence of non-melanoma skin cancer. Int J Cancer 62:165–169

Burits M, Bucar F (2000) Antioxidant activity of *Nigella sativa* essential oil. Phytother Res 14:323–328

Cadenas E, Davies KJA (2000) Mitochondrial free radical generation, oxidative stress, and aging. Free Rad Biol Med 29:222–230

Chakraborty DP, Bhattacharyya P, Roy S, Bhattacharyya SP, Biswas AK (1978) Structure and synthesis of mukonine, a new carbazole alkaloid from Murraya koenigii. Phytochem 17:834–835

Huda FN, Noriham A, Norrakiah AS, Babji AS (2009) Antioxidant activity of plants methanolic extracts containing phenolic compounds. Afr J Biotechnol 8:484–489

Ito C, Thoyama Y, Omura M, Kajiura I, Furukawa H (1993) Alkaloidal constituents of *Murraya koenigii*. Isolation and structural elucidation of novel binary carbazolequinones and carbazole alkaloids. Chem Pharm Bull 41:2096–2100

Singh J, Upadhyay AK, Singh S, Rai M (2009) Total phenolics content and free radical scavenging activity of brassica vegetables. J Food Sci Technol 46:595–597

Mahajan M, Patil M (2004) Antioxidant activity and organic constituents of curry leaf tree (*Murraya koenigii* Linn.). Asian J Microbiol Biotechnol Environm Sci 6:323–324

Ningappa MB, Srinivas L (2008) Purification and characterization of 35 kDa antioxidant protein from curry leaves (*Murraya koenigii* L.). Toxicol in Vitro 22:699–709

Ningappa MB, Dinesha R, Srinivas L (2008) Antioxidant and free radical scavenging activities of polyphenol-enriched curry leaf (*Murraya koenigii* L.) extracts. Food Chem 106:720–728

Rao LJM, Ramalakshmi K, Borse BB, Raghavan B (2007) Antioxidant and radical-scavenging carbazole alkaloids from the oleoresin of curry leaf (*Murraya koenigii* Spreng.). Food Chem 100:742–747

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Rad Biol Med 26:1231–1237

Tachibana Y, Kikuzaki H, Lajis NH, Nakatani N (2001) Antioxidative activity of carbazoles from *Murraya koenigii* leaves. J Agric Food Chem 49:5589–5594

Tachibana Y, Kikuzaki H, Lajis NH, Nakatani N (2003) Comparison of antioxidative properties of carbazole alkaloids from *Murraya koenigii* Leaves. J Agric Food Chem 51:6461–6467

Yu L, Haley S, Perret J, Harris M, Wilson J, Qian M (2002) Free radical scavenging properties of wheat extracts. J Agric Food Chem 50:1619–1624