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Evaluation of Phenolic contents and Antioxidant potential of *Murraya Koenigii* (L) spreng roots

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ABSTRACT

The present study was to evaluate the antioxidant potential of ethanol extract from roots of *Murraya koenigii*. Ethanol extract of roots was used to study their total phenolic and flavonoid contents and antioxidant including radical scavenging of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and nitric oxide. The ethanol extract showed significant results, Total phenolic contents were estimated to be equivalents to 51.23 ± 2.53mg of gallic acid equivalent and total flavonoid contents were 43.58 ± 1.89mg of catechin/g equivalent. The percentage of DPPH and NO scavenging activity increased with increasing various concentration of extract.

INTRODUCTION

Murraya koenigii Linn (Rutaceae) commonly known as Curry patta and Meethi neem, is an aromatic more or less deciduous shrub or a small tree up to 6 m in height found throughout India up to an altitude of 1500 meters. In traditional system of Medicine, it is used as antiemetic, antidiarrhoeal, dysentery, febrifuge, blood purifier, tonic, stomachic, flavouring agent in curries and chetneys (Anonymous, 1998; Prajapati *et al.* 2003).

The plant based medicine has been the stronghold of traditional societies in dealing with health problems (Fransworth, 1994). The World Health Organization has estimated that 80% of the population rely upon traditional medicine for their primary health care needs (Sindhu and Arora, 2012; Kurian, 1995). Plants are a natural source of biologically active compounds known as phytoconstituents (Fransworth, 1994). The phytoconstituents have been found to act as antioxidants by scavenging free radicals, and many have therapeutic potential for free radical associated diseases. Reactive oxygen species (ROS) including hydroxyl radicals, singlet oxygen, hydrogen peroxide and superoxide radicals are frequently generated as by products of biological reaction(Kikuzaki and Nakatani 1993).

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However, these ROS produced by sunlight, ultraviolet light, ionizing radiation, chemical reactions and metabolic processes have a wide variety of pathological effects such as Arthritis, carcinogenesis, DNA damage and various degenerative disorders such as neuro-degenerative diseases, aging and cardiovascular ailments etc. (Osawa, 1994; Noda *et al.* 1997).

MATERIALS AND METHODS

Plant Collection

The plant of *Murraya koenigii* roots were collected during the month of the July 2009 from Chitkara University (Punjab), North India. The plant material was taxonomically identified and authenticated by Dr. H.B. Singh, Head, Raw materials Herbarium and Museum division, with ref. no. NISCAIR/RHMD/ Consult/2010-11/1638/236. The voucher specimen has been deposited in the herbarium section of the Phytochemistry and Pharmacognosy Division, Chitkara College of pharmacy, Chitkara university, Panjab for further reference.

Preparation of Extracts

The dark brown coarse powder 800g was extracted with ethanol in sohxlet apparatus 72 h. The dark brown mass of extract (35.75g) was obtained by concentrating ethanol extract in rotary vacuum evaporator.

Preliminary Phytochemical Studies

The Ethanol extract screened for preliminary phytochemical studies (Sindhu and Arora, 2012). The presence of Alkaloids, phenolic, compounds flavonoids and sterol in extract were observed.

Screening of antioxidant Activity

Total phenolic content

Total phenolic content in the extract was determined using the Folin-Ciocalteu's reagent (FCR) according to Molan et al. 2009. Each sample (0.5 ml) was mixed with 2.5 ml of FCR (diluted 1:10, v/v), and 2 ml of Na_2CO_3 (7.5%, w/v) was added. The absorbance was then measured at 765 nm after incubation at 30ºC for 90 minutes. Results were expressed as gallic acid equivalents (mg of gallic acid/g of dried extract).

Total flavonoid content

The total flavonoid content of ethanol extract was determined using a colorimetric method (Zhishen *et al.*, 1999). Briefly, each sample (0.5 ml) was mixed with 2 ml of distilled water and subsequently with 0.15 ml of a $NaNO_2$ solution (15%, w/v). After 6 minutes, 0.15 ml of an $AlCl_3$ solution (10%, w/v) was added and allowed to stand for 6 minutes, then 2 ml of NaOH solution (4%, w/v) was added to the mixture. Instantaneously, water was added to bring the final volume to 5 ml, and then the mixture was carefully mixed and allowed to stand for another 15 minutes. Absorbance of the mixture was determined at 510 nm versus prepared water blank. Results were expressed as catechin equivalent (mg of catechin/g of dried extract).

DPPH radical scavenging activity

Scavenging activity of diphenyl- 2-picrylhydrazyl (DPPH) radicals of ethanol extract or catechin was measured according to the method reported by Molan *et al.*, 2009, with minor modifications. Assays were performed in 3 ml reaction mixtures containing 2.0 ml of 0.1 mM DPPH-ethanol solution, 0.9 ml of 50 mM Tris-HCl buffer (pH 7.4), and 0.1 ml of deionized H_2O (as control) or test plant extracts. After 30 minutes of incubation at room temperature, absorbances of the reaction mixtures at 517 nm were taken. The inhibitory effect of DPPH was calculated according to the following formula:
Percentage inhibition=[(Absorbance control –Absorbance sample)/Absorbance control] × 100

NO scavenging activity

The scavenging effect of ethanol extract on NO was measured according to Marcocci *et al.*, 1994. Briefly, sodium nitroprusside (5 mM) in phosphate-buffered saline (pH 7.4) was mixed with different concentrations of the test sample (100-1000 mg/ml) and incubated at 25 ºC for 150 minutes. After incubation, nitrite produced from sodium nitroprusside was measured by Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% 1-naphthylethylenediamine dihydrochloride in water). The absorbance of the chromophore that formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with 1-naphthylethyelenediamine dihydrochloride was immediately read at 570 nm. Catechin was used as a positive control. The percentage of NO scavenging was calculated using the following formula:

Percentage inhibition = [(Absorbance control –Absorbance sample)/Absorbance control] × 100

RESULTS AND DISCUSSION

Phenolic and Flavanoid contents

Phenolics are the majority wide spread secondary metabolite in natural drugs. The antioxidant activities of phenols are mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers (Rice-Evans *et al.*, 1995). Total phenolic were estimated to be equivalents to 51.23 ± 2.53 mg of gallic acid. Flavonoids are a class of plant phenolics with prevailing antioxidant properties (Pietta, 2000). Total flavonoid contents were 43.58 ± 1.89 mg of catechin/g of dried roots extract, respectively.

Free radical scavenging activity

The DPPH antioxidant assay is based on the ability of DPPH a stable free radical, to decolorize in the existence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. The percentage of free radical scavenging activity of alcoholic extract and ascorbic acid determined at different concentrations (10, 20, 40, 80 and 160) and at higher concentration it was found to be 54.92% and 62.24% for ascorbic acid respectively as shown in figure 1. The absorbance was decreased as concentration of extract increasing as shown in figure 2.

Table. 1: Free radical scavenging activity of ethanol extract.

Sr. No	Concentration (µg/ml)	Absorbance (517nm)		DPPH % inhibition	
		Ethanol Extract	Ascorbic acid	Ethanol Extract	Ascorbic acid
1.	10	0.532	0.503	12.79	17.38
2.	20	0.520	0.465	14.75	23.79
3.	40	0.450	0.405	26.95	33.60
4.	80	0.380	0.314	37.35	44.10
5.	160	0.275	0.225	54.92	62.24

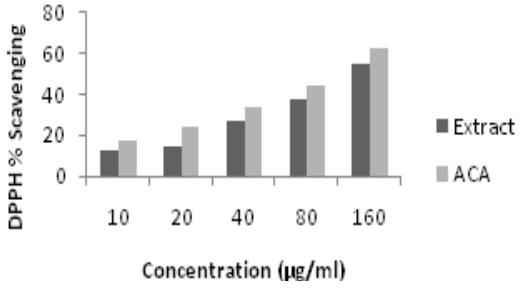

Fig. 1: DPPH % scavenging activity of Ethanol extract of *Murraya koenigii* roots at different concentrations (µg/ml) comparison with standard (Ascorbic acid). Extract (Ethanolic), ACA= Ascorbic acid

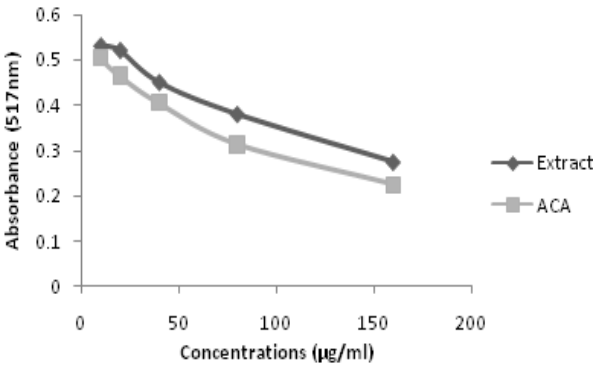

Fig. 2: Absorbance of DPPH scavenging activity of Ethanol extract of *Murraya koenigii* roots at different concentrations (µg/ml) comparison with standard (Ascorbic acid). Extract (Ethanolic), ACA= Ascorbic acid.

NO scavenging activity

The NO scavenging activity was observed by various concentrations (10.60%, 17.45%, 30.30%, 35.60% and 55.30% at extract concentrations of 100, 200, 400, 800 and 1000 µg/ml, respectively). This is shown in Figure 3. Incubation of a sodium nitroprusside solution in phosphate buffered saline at 25 ºC for 150 minutes resulted in linear time-dependent nitrite production, which was reduced by ethanol extract in a concentration-dependent manner. The absorbance was decreased as concentration of extract increasing as shown in figure 4.

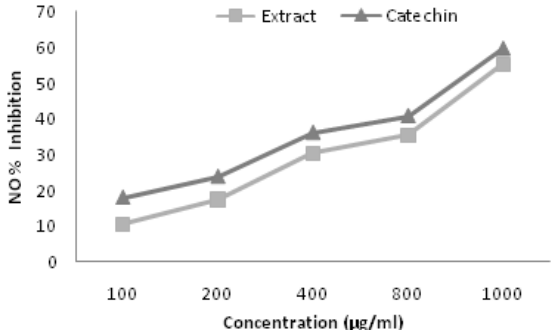

Fig. 3: NO % scavenging activity of Ethanol extract of *Murraya koenigii* roots in different concentrations (µg/ml) comparison with standard (Catechin), NO = Nitric oxide.

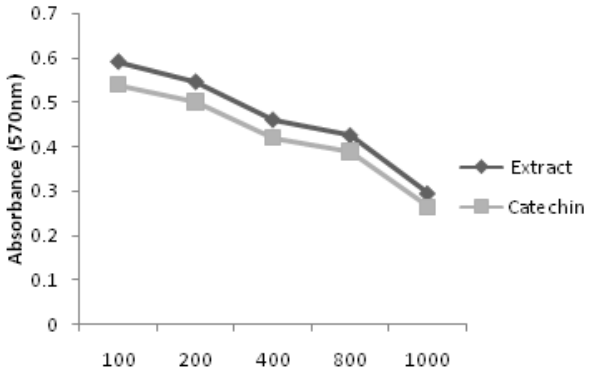

Fig. 4: Absorbance of NO scavenging activity of Ethanolic extract of *Murraya koenigii* roots at different concentrations (µg/ml) comparison with standard (Catechin), NO = Nitric oxide.

CONCLUSION

The alcohol extract of roots of *Murraya koenigii* had shown very significant total phenolic content, total flavanoids content and free radical scavenging activity by DPPH and NO methods. The results concluded that the extract have various types of phytoconstituents which are the source of antioxidants of natural origin that could have great importance as therapeutic agents for biological system.

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REFERENCES

Anonymous. The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products. Publication & Information Directorate, New Delhi : CSIR, 1998;446-448.

Farnsworth NR: Ethnopharmacology and Drug Development. In: Ethnobotany and the search for new drugs, Wiley, Chichester (Ciba Foundation Symposium 185); 1994.

Kikuzaki H, Nakatani N. Antioxidant effects of some ginger constituents. Journal of Food Science 1993; 58(6): 1407-1410.

Kurain JC: In : Plants That Heal, Oriental Watchman Publishing House, Pune, India. 1995; 296;

Marcocci L, Maguire JJ, Droy-Lafaix MT, Packer L. The nitric oxide-scavenging properties of Ginkgo biloba extract EGb 761. Biochem Biophys Res Commun, 1994; 201:748-755.

Molan AL, Flanagan J, Wei W, Moughan PJ: Selenium-containing green tea has higher antioxidant and prebiotic activities than regular green tea. Food Chemistry, 2009;114:829-835.

Noda Y, Anzai-Kmori A, Kohono M, Shimnei M, Packer L. Hydroxyl and superoxide anion radical scavengingactivities of natural source antioxidants using the computerized JES-FR30 ESR spectromoter system. Biochem. Mol. Biol. Inter. 1997; 42 : 35-44.

Osawa T. Postharvest biochemistry. Novel neutral antioxidant for utilization in food and biological systems. Japan: Japan Scientific Societies Press; 1994; 241-251.

Pietta DG. Flavonoids as antioxidants. J Nat Prod. 2000; 63:1035-1042.

Prajapati ND, Purohit SS, Sharma AK, Kumar T. A Handbook of Medicinal Plants. Jodhpur: Agrobios, 2003; 352-353.

Rice-Evans CA, Miller NJ, Bollwell PG, Bramley PM, Pridham JB: The Relative Antioxidant Activities of Plant-Derived Polyphenolic Flavonoids. Free Radical Res. 1995; 22:375-383.

Sindhu RK, Arora S. Phytochemical and Pharmacognostical studies on *Murraya koenig* L . spreng roots. Drug Invention Today. 2012; 4:325-336.

Zhishen J, Mengcheng T, Jianming W: The determination of flavonoid contents in Mulberry and their scavenging effects on superoxide radicals. Food Chem, 1999; 64:55-59.

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