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Assessment of Antioxidant Activity of Leaves of *Murraya koenigii* Extracts and it's Comparative Efficacy Analysis in Different Solvents

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Abstract

There are many phytochemicals which help the human body in a number of ways. Phytochemicals are non-nutritive plant compounds which have protective or disease preventive properties. Plants produce these chemicals to protect themselves, but recent research shows that many phytochemicals have the potential to provide protection against human diseases. Extensive researches on *Murraya koenigii* (curry leaf) showed that this plant is well accepted for treatment among different communities for curing several disorders. *M. koenigii* have been found to possess a number of medicinal properties like anti-diarrheal, antiulcer, anti-diabetic, antioxidant, cytotoxic activity and is also screened by scientific groups for antimicrobial potential as well. It is well-recognized that different solvents have the ability to extract different types of phytochemicals on the basis of their polarity; henceforth, the biological activity of the extracts can be varied. In the present study, efforts were done to analyze the phyto-components of curry leaves by using different solvent systems and then comparative evaluation of its antioxidant activity in both young and old leaves was done.

Keywords: Antioxidant, Medicinal, Phytocompounds, Cyotoxicity

INTRODUCTION

Since time immemorial, plants have been an inseparable part of human civilization. Plants and their parts have been used in various forms either as food, fodder, timber or as medicines. According to World Health Organization, a large proportion of the world uses plant-based medicines (Phytomedicines) for their primary health care [1]. In a country like India, different existing medicinal usage systems like Unani, Ayurveda, Siddha and local health traditions are focused on using plant based chemicals for treating several human and animal diseases [2]. Medicinal plants have a number of bioactive compounds which have therapeutic properties and help in improving health. Besides being a good source of anti-infective agents, they are also economical and have lesser known side effects [3].

Leaves of *Murraya koenigii* are a popular leaf-spice used in very small amounts for their characteristic aroma because of the presence of volatile oil and also their potential to aid digestion. "Let food be your medicine and let medicine be your food" justifying this saying, several scientists and medical researchers have shown immense interest in the area of herbal medicines as they identify the therapeutic potential of these natural products or folk medicines [4].

Antioxidants are compounds that the body uses to protect themselves from free radicals which are generated during oxidation reactions in the metabolism. These active species initiate a series of reactions like DNA oxidation, protein oxidation or lipid peroxidation which in turn, thus harm cell metabolism. Plant phenolics especially, tannins, flavonoids and phenolics are familiar compounds with remarkable antioxidant activity [5]. Hence the research has been focused on identification of plant based antioxidants, which may scavenge free radicals and may exhibit protective effects.

MATERIALS & METHODS

1. Collection of plant material

The new and old leaves of *Murraya koenigii* were collected in zipper bags from local gardens of Thatipur, Gwalior and authenticated by Professor of Botany in Amity University, Gwalior. Later washed two times with tap water and they were dried under shade at ambient temperature for 6-7 days.

2. Preparation of Plant Extracts

Dried leaves (both young and old) were ground to fine powder using mortar and pestle. 500 mg dried powder was soaked in 1 ml of different solvents such as petroleum ether, DMSO, distilled water, isopropyl alcohol, acetone, ethanol and methanol for 48 hours. All the chemicals and reagents that were used were of analytical grade. The supernatant was then collected by centrifugation at 3000 rpm.

3. Preliminary Phytochemical Analysis

The extracts were concentrated and several chemical tests were performed to test the presence of various phytochemicals. These qualitative tests were performed according to the protocol devised by Harborne *et al*., 1973 with certain modifications [6].

4. Antioxidant Assay

4.1. Total Phenols

Total phenolic content was determined by Folin ciocalteau reagent method [7]. An aliquot of each extract prepared in different solvent system (0.5ml of 1:10 mg/l) or Gallic acid (standard phenolic compound) was added to Folin ciocalteau reagent (5ml 1:10 diluted with distilled water) and 4ml of 1M solution of Na_2CO_3 was added to it. The mixture was incubated at room temperature (37°C) for 30 minutes and absorbance was measured against blank at 710 nm. Total phenolic contents were expressed as mg Gallic

acid equivalent (GAE)/gm dry weight. All experiments were performed in triplicates.

4.2 Hydrolysable Tannins (Gallotanin)

1.5 ml of plant extract was taken and added to 0.5 ml of saturated potassium iodide then the reaction mixture was incubated at 37°C for 40 minutes and absorbance was measured at 550 nm with saturated potassium iodide as blank.

4.3 Hydrogen Peroxide Scavenging Activity

1ml of plant extract was taken and added to 2ml (20mm) H_2O_2 in PBS which was then incubated for 10 minutes and absorbance was taken at 230 nm.

RESULTS & DISCUSSION

Curry leaf (*Murraya koenigii*) belongs to Rutaceae family and is an important leafy vegetable. The dried leaves of this plant are also used as a flavouring agent in cooking in India. It is also used in many of the Indian ayurvedic and unani medicines.

A variety of chemical compounds from every part of the plant have already been isolated. The major compounds responsible for its characteristic flavor are P-elemene, P-caryophyllene, P-gurjunene and O-phellandrene. The plant is rich in carbazole alkaloid. The plant poses tonic and stomachic properties. *Murraya koenigii* is credited with numerous chemical compounds that interact in a complex manner to stimulate its pharmacodynamic response [8].

The human body experiences several cellular and metabolic injuries daily either due to external factors such as environmental pollution or pathogen invasion or due to the internal metabolism. This stress is associated with the generation of reactive free radicals within the body that causes oxidation of biomolecules and thus renders weakened immune system [9-11]. To combat the ill effects of free radicals in the human body, supplementation of antioxidants in a diet could be a long-term strategy to prevent life style-induced non-communicable diseases such as hypertension, cardiovascular diseases and cancers [12-13]. Addition of fresh curry leaves in the diet of albino rat showed an alteration in peroxidation level to a remarkable extent [14]. Thus, antioxidant activity of the plant with potent bioactives has been reported by a number of workers.

Preliminary phytochemical screening of young and old leaves in different solvent extracts showed the presence of saponin, tannins, steroids, alkaloids and flavonoids.

Different solvents were chosen to perform antioxidant assays as phenols are generally potent inhibitors of free radicals and hence better antioxidant agents were extracted in different solvents at varied concentrations as depicted in Table no. 3.

The Folin-Ciocalteau phenol reagent is used to have a quantitative estimation for the concentration of phenolic compounds present in the plant extracts. The result of the total phenol assay (Table no. 3) showed that the phenolic content of old leaves in methanol extract and in the DMSO extract of young leaves is higher in comparison to extracts of other solvents. Wong *et al.* (2006) reported a similar trend where the total polyphenol of aqueous extract of curry leaves was also higher when compared to leaf extracts of other plants [15].

Table no. 1 Phytochemical analysis of old leaves in different solvents:

Solvent	Saponin	Tannins	Steroid	Alkaloids	Flavonoids
Ethanol	-	+	+	-	-
Methanol	-	+	+	-	-
Acetone	-	+	+	-	-
IPA	-	+	+	-	-
DMSO	-	+	+	-	-
Distilled water	-	-	+	-	+
Petroleum ether	-	+	+	-	-

+ indicates presence and – indicates absence

Table no. 2 Phytochemical analysis of young leaves in different solvents:

Solvent	Saponin	Tannins	Steroids	Alkaloids	Flavonoids
Ethanol	-	+	+	-	-
Methanol	-	+	+	-	-
Acetone	-	+	+	-	-
IPA	-	+	+	-	-
DMSO	-	+	+	-	-
Distilled water	-	-	+	-	+
Petroleum ether	-	+	+	-	+

+ indicates presence and – indicates absence

Table no. 3. Absorbance of Total Phenol Assay of *Murraya koinigii* leaves in different solvents:

Solvent	Abs_{710nm} (old leaves)	Abs_{710nm} (young leaves)
Ethanol	0.634	0.787
Methanol	0.640	0.867
Acetone	0.271	0.840
IPA	0.434	0.535
DMSO	0.326	0.908
Distilled water	0.434	0.632
Petroleum ether	0.024	0.230

Table no. 4 Concentration of tannins in *M. koiengii* leaves in different solvents:

Solvent	Concentration in mg equivalent to catechin (old leaves) in mg	Concentration in mg equivalent to catechin (young leaves) in mg
Ethanol	21.0	45.7
Methanol	36.3	23.9
Acetone	34.7	23.3
IPA	46.3	46.7
DMSO	4.5	4.94
Distilled water	6.5	2.3
Petroleum ether	5.7	0.052

Table no. 5 Hydrogen peroxide scavenging activity of *M. koiengii* leaves in different solvents:

Solvent	% scavenging activity (old leaves)	% scavenging activity (young leaves)
Ethanol	103.53%	10.19%
Methanol	0.091%	8.21%
Acetone	151.58%	10.10%
IPA	4.29%	9.02%
DMSO	69.53%	7.32%
Distilled water	56.18%	2.62%
Petroleum ether	81.52%	72.23%

As phenolic components known as primary antioxidants derived from plants are polar in nature and hence dissolved in polar solvents [16].

Tannins are complex secondary metabolite having various medicinal properties [17]. The amount of hydrolysable tannin in leaf extract of *Murraya koinigii* was found maximum in IPA for both old and young leaves, 46.3mg GAE/g and 46.7mg GAE/g respectively (Table no. 4). The Hydrogen per oxide scavenging activity was found maximum in acetone for old leaves (151.58%) and for young leaves in petroleum ether (72.23%).

Although many studies support that total phenols and flavonoids are prime factors that contribute to the total antioxidant potential of several vegetables and fruits [18-20], our observations that scavenging potential as well as presence of phenols in young and old leaves add precision to the available knowledge in this area of work. In addition, the observations are likely to sensitize further basic physiological research on the possible association of process of aging and enhance their antioxidant activity. Reports are also available on analysis and isolation of antioxidant vitamins and active carbazole alkaloids from fresh leaves of *M. koenigii* [21-23] but the present work was first in its form to report the comparative study of antioxidant activity of extracts from younger and older leaves of *M. koenigii* in different solvent systems.

CONCLUSION

The available pre-clinical data in the literature indicates the remarkable pharmacological activities of *M. koenigii* which makes it suitable to be utilized to improve the treatment of variety of diseases. The crude extract of the plant have enormous medical applications, yet the extensive study using animal models and clinical trials to explore the exact molecular mechanism of action, efficacy analysis against microbes and toxicity assays should be carried out in quest of lead compounds from natural repository for effective drug development.

Extensive research and wide spread availability of *M. koenigii* in India thus makes it a suitable candidate for further pre-clinical and clinical trials. In future study, the active principle compounds from curry leaf needs to be isolated and evaluated in scientific manner.

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