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Full Length Research Paper

Total phenolic (flavonoids) contents and antioxidant capacity of different *Murraya koenigii* (L.) Spreng. extracts

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In the present study, antioxidant activities of the three plant parts (stem, leaf and callus) were investigated. Ethanolic extracts of stem, leaf and callus powder was characterized by the method described by (Hatano et al., 1988) in which stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical solution was used. In this paper, it is found that all of the *in vitro* and *in vivo* extracts have remarkable antioxidant activities. The IC_{50} (inhibition concentration 50) of the ethanolic extracts of M Koenigii callus-stem-leaf were 47-78, and 116 µg/ml respectively. The total phenolics and flavonoid content of the *Murraya koenigii* callus cultures were also measured. Among them *in vitro* callus showed higher antioxidant activity, due to phenolics and flavonoid content. Therefore, the research clearly indicates that *M. koenigii* callus is exceptionally advantageous for human health.

Key words: *In vitro* propagation, *Murraya koenigii* (L.) spreng, ethanolic extracts, IC_{50}.

INTRODUCTION

A whole range of plant derived dietary supplements, phytochemicals and pro-vitamins that assist in maintaining good health and combating disease are now being described as functional foods, and nutraceuticals. A wealth of information and scientific evidences are rapidly accumulating that shows the beneficial effects of a wide variety of food components on human health. In this context, M. *koenigii in vitro* and *in vivo* extracts are immensely valued not only for their nutritional content but also for their potential heath functionality against various degenerative diseases such as cancer, cardiovascular, cataract, diabetes, and neurodegenerative diseases like Alzheimer's and Parkinson's (Anonymous, 1988). The protection that fruits provide against these diseases has been attributed to the various antioxidants present in them (Baral and Kurmi 2006). The major group of phytochemicals that may contribute to antioxidant capacity of fruits includes polyphenols, carotenoids, and the traditional antioxidant vitamins such as vitamin C and E. The fruit polyphenols are the most important group of natural antioxidants because of their diversity and extensive distribution, and they possess the ability to scavenge both active oxygen species and electrophiles.

In recent years, with enhanced awareness of the importance of antioxidant compounds in health and disease, considerable attention has been devoted to aromatic medicinal plants with high antioxidant properties (Kong et al., 2010; Mohamed et al., 2010).

M. koenigii (L.) Spreng. commonly known as curry leaf tree belongs to family Rutaceae. The plant oil contains about 39 compounds of which the major are 3-carene (54.2%) and caryophyllene (9.5%), which are being used to control diabetes, leprosy, gastrointestinal diseases, vomiting, diarrhoea, skin diseases and are also act as a fixative for a heavy type of soap perfume (Baral and Kurmi, 2006.). The leaves are mainly employed as flavoring agent in curries due to the presence of aroma components like non terpenoids acyclic β ketones and are also the important source of vitamin A and C. Its aqueous extract is hyperglycemic (Kesari et al., 2005). The root extract also attributes many medicinal properties like anti-bacterial, anti-inflammatory and anti-feedant etc. which have been used to relieve pain associated with kidney (Anonymous, 1988).

A number of secondary metabolites have previously been reported in this plant including phenolic, tannins

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Abbreviations: BAP; - 6-benzyladeninopurine, **MS;** Murashige and Skoog's medium, **DPPH-1;** 1-diphenyl-2-picrylhydrazyl.

flavonoids,alkaloids. Moreover, higher content of total mahanimbine and murrayanol have been attained in *M. koenigii* mature leaf callus culture as a result of the impact of different combinations of growth regulators. *M. koenigii* stem, leaf and callus extracts demonstrated activity against some bacteria and fungi prevalent in dermatology, so it can be considered as an effective antimicrobial agent to treat numerous diseases (Ziyyat et al., 1997). No information is available about the stimulation effects of plant growth regulators on antioxidant activity of *M. koenigii* callus cultures.

The aim of this study was to develop conditions for callus cultures of *M. koenigii* manipulating the concentrations and combinations of plant growth regulators, and determination of the effect of media composition on total phenolics and flavonoids content together with the related antioxidant activity of induced calli.

MATERIALS AND METHODS

Chemicals

All the chemicals and growth regulators were used are analytical grade and purchased from Hi Media Pvt. Limited, Mumbai, India.

Explant source

Mature leaf of *M. koenigii* (L.) Spreng were collected in the month of July, from the Botanical garden, University of Rajasthan, Jaipur. Leaves washed in running tap water for about 5 min and then kept in teepol 1% (v/v) and 0.02% (w/v) solution of Bavistin fungiside for about 2 min followed by rinsing with sterilized double distilled water for 5 to 6 times to remove all traces of sterillant. They were then surface sterilized with 2% sodium hypochlorite (NaOCl; v/v) and 0.1% mercuric chloride ($HgCl_2$; w/v) for 2 min respectively and then rinsed with double distilled water at least thrice.

For callus culture initiation, full strength MS (Murashige and Skoog's,1962) was taken as basal medium containing 3% sucrose 0.8%. Agar supplemented with various cytokinins and auxin like 2,4-D and Kn. The media were adjusted to p^H 5.8 ± 0.2 and autoclaved at 1.1 kg/cm^2 for 20 min.

Preparation of plant extracts

Callus cultures derived from aromatic mature leaves as well as *in vivo* leaf and stem materials (used as control) were air dried at room temperature and ground in a mortar. The extracts were prepared using the modified method of (Matkowski and Piotrowska, 2006). Briefly, 0.5 g of the dried powder from each sample was refluxed with ethanol in a water bath at 45°C for 3 h. The extracts were filtered (Whatman filter paper No. 1) and filtrates were dried under vacuum at 40°C. The extraction was repeated twice. The resulting residue was re-dissolved in ethanol and used for the determination of phenolics, flavonoid contents and antioxidant activities.

Estimation of total phenolics content

The total phenolic content of *in vitro* and *in vivo* extracts was determined by Folin- Ciocalteu method (Swain and Hillis, 1959). The amount of total phenolics and tannins were calculated as g gallic acid quivalents (GAE) from the calibration curve (Makkar et al., 2007).

Estimation of total flavonoid content

The total flavonoid content of sample extracts was determined by the use of a slightly modified colorimetric method described by (Zhishen and Jianming, 1999). A 0.5 mL (1 mg/mL of ethanol) extract was mixed with 2 mL of distilled water and subsequently with 0.15 mL of 5% $NaNO_2$ solution. After 6 min of incubation, 0.15 mL of 10% $AlCl_3$ solution was added and allowed to stand for 6 min, and then 2 mL of 4% NaOH solution was added to the mixture. Immediately distilled water was added to bring the final volume to 5 mL, and then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm versus prepared water blank. Rutin was used as standard compound for the quantification of total flavonoid. All the values were expressed as g rutin equivalent (RE)/ 100 g of extract.

Measurement of antioxidant activities

The antioxidant activity of the ethanolic extracts was determined on the basis of their scavenging activity of the stable 1, 1- diphenyl-2-picryl hydrazyl (DPPH) free radical. DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging, activity in chemical analysis. 1 ml of each solution of different concentrations (1-500µg/ml) of the extracts was added to 3 ml of 0.004% ethanolic DPPH solution. After 30 min, the absorbances of the preparations were taken at 517 nm by a UV spectrophotometer which was compared with the corresponding absorbance of standard ascorbic acid concentrations (1-500 µg/ml). The method (Hatano et al., 1988) was used to measure the absorbance with some modifications. Then the % inhibition was calculated by the following equation:

% radical = (absorbance of blank-absorbance of scavenging activity sample)/(absorbance of blank) × 100

Table 1. Total phenolics,and flavonoids contents *in vitro* and *in vivo* extracts.

Extracts	Total phenolics	Total flavonoids
Callus	11.6 ± 20	50.0 ± 26
Stem	10.7 ± 34	35.1 ± 23
Leaf	10.0 ± 20	30.5 ± 34

From calibration curves, obtained from different concentrations of the extracts, the IC_{50} (Inhibitory concentration 50%) was determined. IC value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals (Gupta et al., 2003).

Procedure

1. Six (6) test tubes were taken to make aliquots of 6 conc. (1, 5, 10, 50, 100 and 500 µg/ml) with the samples.
2. *In* vitro and *in vivo* plant extracts and ascorbic acid were weighed accurately and dissolved in ethanol to make the required concentrations by dilution technique. Here ascorbic acid was taken as standard.
3. DPPH was weighed and dissolved in ethanol to make 0.004% (w/v) solution. To dissolve homogeneously magnetic stirrer was used.
4. After making the desired concentrations 3 ml of 0.004% DPPH solution was applied on each test tube by pipette.
5. The room temperature was recorded and kept the test tubes for 30 min in light to complete the reactions.
6. DPPH was also applied on the blank test tubes at the same time where only ethanol was taken as blank.
7. After making the desired concentrations 3 ml of 0.004% DPPH solution was applied on each test tube by pipette.
8. The room temperature was recorded and kept the test tubes for 30 min in light to complete the reactions.
9. After 30 minutes, the absorbances of each test tube were taken by a UV spectrophotometer.
10. IC_{50}'s were measured from % Inhibition *vs.* Conc. graphs.

RESULTS AND DISCUSSION

Total phenolics and flavonoid content

Plant phenolics present in the callus have received considerable attention because of their potential biological activity. Phenolic compounds such as flavonoids and phenolic possess diverse biological activities including anti-inflammatory, anti-carcinogenic, and antidiabetic activities. These activities might be related to their antioxidant activity (Chung et al., 1998). The contents of total phenolics and flavonoids in certain callus stem and leaf extracts are estimated and the data presented in Table 1. The total phenolic content varied significantly among the different *in vitro* and *in vivo* extracts between 10.0. and 11.6 GAE g/100 g extract. The extracts of murraya koenigii callus (11.6), stem. (10.7), and leaf. (10.0). *M. koenigii* callus (11.6) recoded higher total phenolic content. However, moderate levels of phenolics were reported in stem and leaf.

The results obtained in the quantitative analysis flavonoids (Table 1) revealed that all samples have considerable amounts of total flavonoid content. However, higher levels of flavonoids was observed in *M. koenigii* callus (50.0 RE g/100 g extract, respectively) followed by stem and leaf (35.1 and 30.5. RE g/ 100 g extract, respectively). The highest antioxidant activity reported for the said extracts is due to the presence of these bioactive constituents.

The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994). The importance of the antioxidant constituents of plant material in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers, and consumers. It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when ingested daily up to 1.0 g from a diet rich in fruits and vegetables (Tanaka et al., 1988).

1-diphenyl-2-picrylhydrazyl (DPPH) stable free radical scavenging activity

In the present study, ethanolic extracts of the two varieties of the explant of the *M. koenigii* callus showed potential free-radical scavenging activity. The antioxidant activities of the individual compounds, present in the extracts may depend hydroxyl or methoxyl groups, flavone hydroxyl, keto groups, free carboxylic groups and other structural features (Patt and Hudson, 1990).

Murraya koenigii (Callus)

From Figures 1 and 2, it is found that IC_{50} of the callus extract of *M. koenigii* (callus) is 47µg/ml which indicates the remarkable antioxidant activity of the extract.

Murraya koenigii (stem)

From Figures 3 and 4, it is found that IC_{50} of the stem

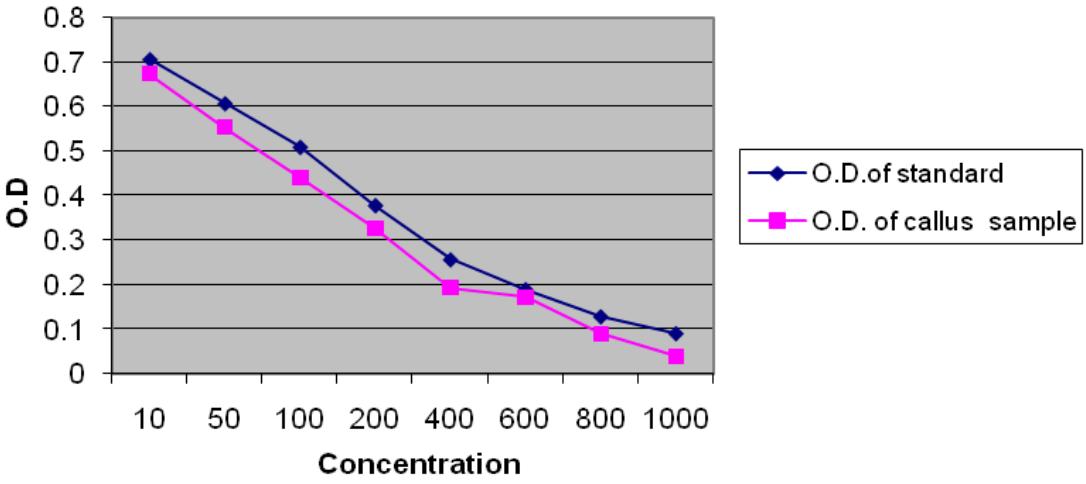

Figure 1. DPPH scavenging Assay of the callus extract of *M. Koenigii* compared with standard ascorbic acid.

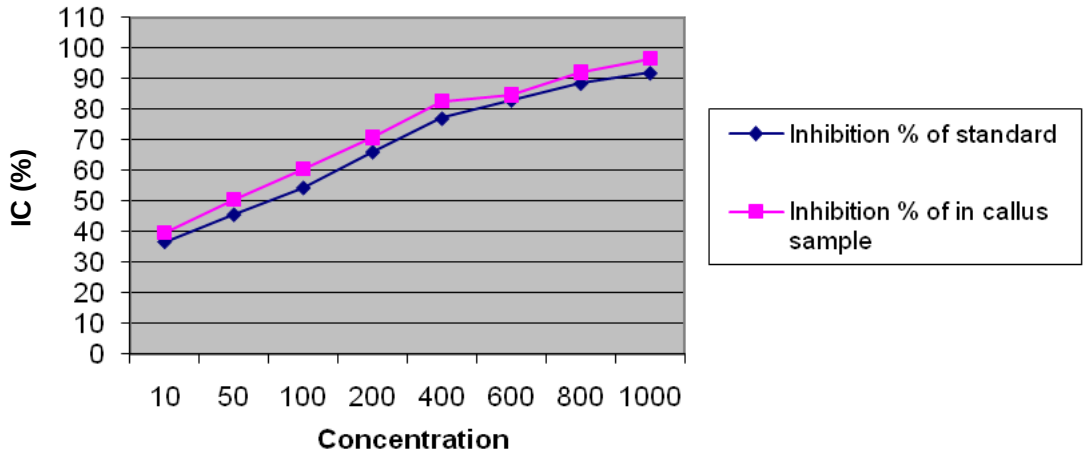

Figure 2. Evaluation of IC_{50} of the callus extract of *M. Koenigii* with standard ascorbic acid.

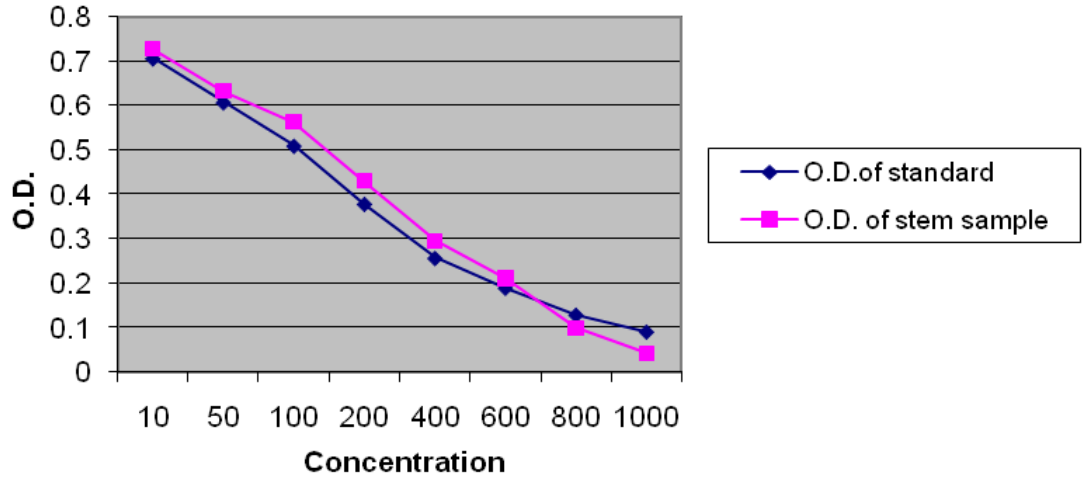

Figure 3. DPPH scavenging Assay of the stem extract of *M. Koenigii* compared with standard ascorbic acid.

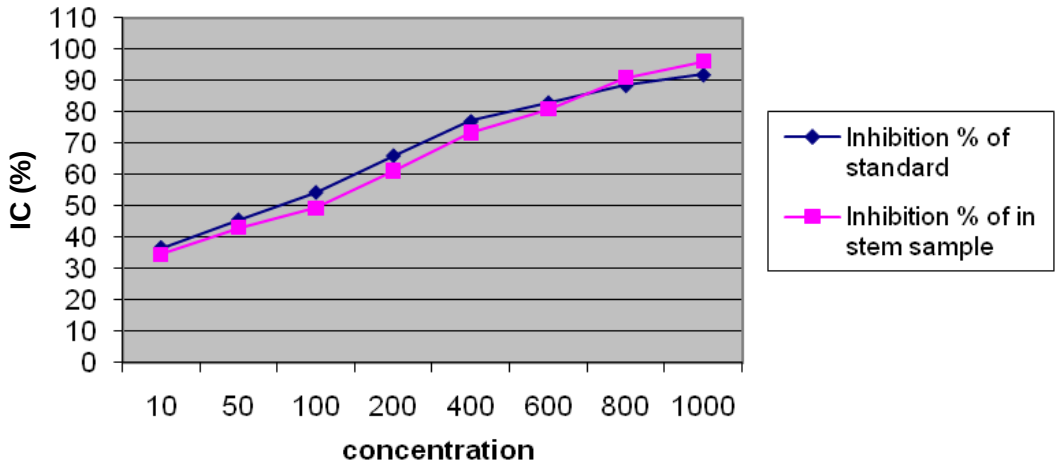

Figure 4. Evaluation of IC_{50} of the stem extract of *M. Koenigii* and standard ascorbic acid.

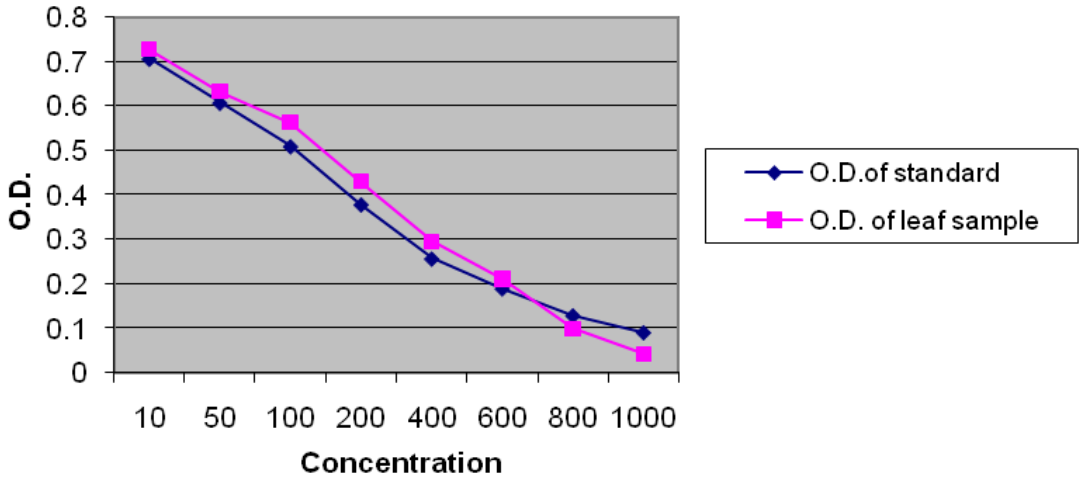

Figure 5. DPPH scavenging Assay of the leaf extract of *M. Koenigii* compared with standard ascorbic acid.

extract of *M. koenigii* 78µg/ml which indicates that low level of antioxidant activity. The antioxidant activity may be due to the presence of phenolic hydroxyl or methoxyl groups, flavone hydroxyl, keto groups, free carboxylic groups and other structural features.

***Murraya koenigii* (leaf)**

From Figures 5 and 6, it is found that IC_{50} of the leaf extract of *M. koenigii* 116µg/ml which indicates that there is very a low level of antioxidant activity.

Conclusion

The result of the present study showed that the extract of *M. koenigii* explants and their respective callus had the potential to induce antioxidant activity and phenolic metabolites, but it seemed that the of cells in callus cultures could be a reason for expressing these desired secondary metabolites in lower ranges. Nevertheless, this work provides basic information for massive production of phenolic compounds as antioxidants in *M. koenigii* and indicated the ability to utilize plant biotechnology techniques towards development of desired bioactive metabolites *in vitro* culture as an alternative way to avoid using aromatic medicinal plants in pharmaceutical purposes.

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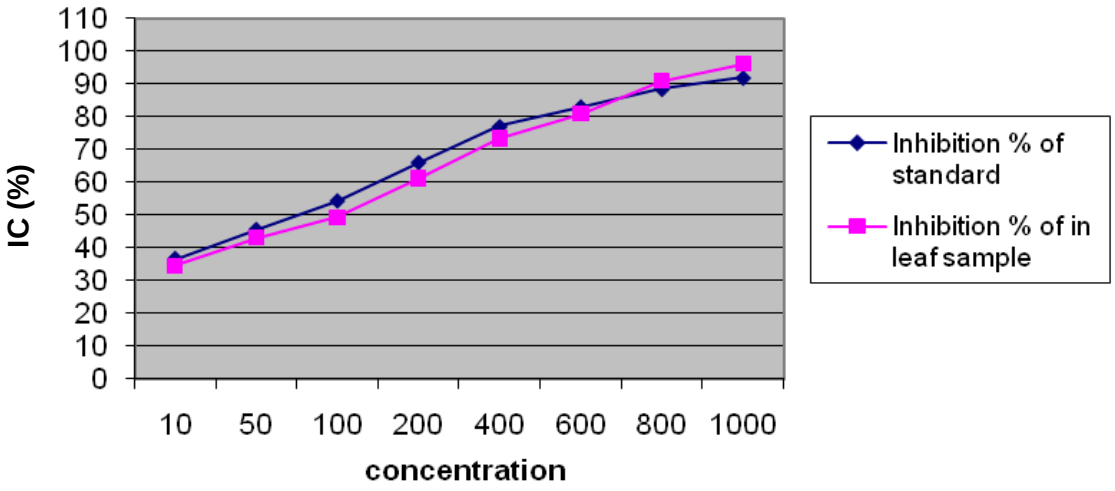

Figure 6. Evaluation of IC_{50} of the leaf extract of *M. Koenigii* and standard ascorbic acid.

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