ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

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Investigation of antioxidant and antidiabetic activities of *Murraya koenigii* leaves methanolic and aqueous extract by *in-vitro* methods

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Article History:

Received on: 11.09.2018
Revised on: 30.09.2018
Accepted on: 01.10.2018

Keywords:

Antioxidant,
Antidiabetic,
Free radical scavenging activity,
Yeast glucose uptake rate

ABSTRACT

Phytochemical investigation of aqueous (AEMK) and methanolic (MEMK) extracts of *Murraya koenigii* were screened for the presence of phytoconstituents like phenolics, flavonoids, saponins, alkaloids, and glycosides. The antioxidant and antidiabetic activity of methanolic and aqueous extracts of *Murraya koenigii* leaves was estimated by DPPH radical scavenging assay and yeast glucose uptake method. Total phenolic and total flavonoid contents were quantitatively estimated. Total phenolics content in AEMK and MEMK were 2.214 mg/g and 2.591 mg/g respectively. Total flavonoid contents in AEMK and MEMK were 2.737 mg/g and 5.248 mg/g correspondingly. AEMK and MEMK have shown the remarkable DPPH free radical scavenging activity. Glucose uptake rate was dependent on the concentration of extracts and decreased the extracellular glucose concentration. In 300 µL concentration, methanolic and aqueous extracts of *Murraya koenigii* exhibited the highest antidiabetic activity in the yeast glucose uptake method. Thus, curry leaves showed a good natural source of antioxidative and antidiabetic compounds to prevent oxidative damage and to reduce blood sugar level.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v9i4.1703

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INTRODUCTION

Murraya koenigii Linn is an aromatic pubescent shrub or small tree generally known as "*Kadhipatta*" in India and as a common synonym as Curry leaves. It contains several bioactive compounds and essential oil which contribute various pharmacological effects (Rajendran *et al.,* 2014). Traditional review of literature describes its potential role in household remedy for various disorders. So the whole plant is considered to be a source of vit-

(Gill *et al.,* 2014). Reactive oxygen species (ROS) including hydroxyl radicals, singlet oxygen, hydrogen peroxide and superoxide radicals are frequently generated as byproducts of the oxidative reaction and oxidative stress is an imbalance be- amins, flavonoids, and phenolics tonic, antidiar- rheal, febrifuge, blood purifier and as stomachic

tween ROS production and ability to readily detox- ify the generated free radicals in the body (Better- idge 2000). Antioxidants are acting through either inhibition of oxidative reaction or scavenging ROS to minimize oxidative stress in the body (Phatak *et al.,* 2015). So the study was aimed to screen phyto- chemical constituents, to ascertain *in-vitro* antioxi- dant and antidiabetic activities; and to assess the quantitative determination of phenolics and flavo- noids content of *Murraya koenigii* leaves aqueous and methanolic extracts.

MATERIAL AND METHODS

Drug and chemicals

Methanol (Loba-Chemie Pvt. Ltd, India), 2, 2'-di- phenyl-2-picrylhydrazyl (DPPH) (Sigma-Aldrich Ltd, India), aluminium chloride (Loba-Chemie Pvt. Ltd, India), folin-ciocalteu reagent (Loba-Chemie Pvt. Ltd, India), sodium carbonate (Loba-Chemie Pvt. Ltd, India), ascorbic acid (Loba-Chemie Pvt.

Ltd, India), gallic acid (Loba-Chemie Pvt. Ltd, India), rutin (Loba-Chemie Pvt. Ltd, India) and metronidazole tablet (J. B Chemicals, India) were purchased. All reagents and chemicals used were of analytical grade and stored in a refrigerator at +4˚C. The reagents were equilibrated at room temperature for 30 minutes before the start of analysis.

Collection and authentication of plant material

Leaves of *Murraya koenigii* were collected from the local area of Karad in Maharashtra, India (17.2760° N, 74.2003° E), certified and authenticated by Department of Botany, M. S. Shinde Mahavidyalaya, Tisangi, Kolhapur, India. The plant specimen voucher no: V03 (Ref: MHST/2016-17/28) of the plant was deposited in the herbarium. Fresh leaves were purchased from the local market of Karad, washed under tap water thoroughly; dried under shade and powdered by using a mechanical grinder.

Preparation of *Murraya koenigii* leaves extract

Methanolic and aqueous extracts of *Murraya koenigii* leaves were prepared by soxhletation method. About 50 g of shade-dried leaves powder of *Murraya koenigii* was packed in a cloth bag and placed in the thistle of Soxhlet apparatus. In Soxhlet apparatus, hot continuous extraction process was continued for 4 days till the dark brown colour of aqueous extract turned to pale yellow while in case of methanolic extract, the appearance of dark green to colourless. Collected extracts were concentrated in a vacuum rotary evaporator at Govt. College of Pharmacy, Karad, Maharashtra, India and dried by evaporating in a hot air oven at 45°C. Percentage yield of methanolic extract of *Murraya koenigii* (MEMK) and aqueous extract of *Murraya koenigii* (AEMK) was calculated with respect to the total quantity of powder used for the extraction.

Phytochemical investigation of *Murraya koenigii* leaves extract

Qualitative screening tests were assessed for various phytochemical compounds present in AEMK and MEMK (Singh *et al.,* 2013).

a) Test for flavonoids: Alkaline reagent test

b) Test for phenolics: Lead acetate test

c) Test for saponins: Froth test

d) Test for alkaloids: Hager's test

e) Test for glycosides: Keller Killiani test

Total phenolics content estimation

Folin-Ciocalteu method was used to determine the total phenolics content of AEMK, MEMK and Gallic Acid (GA) in the method of (Singleton *et al.,* 1965). For quantitative analysis of phenolics, GA in the different range of concentrations was determined and a calibration curve was drawn to assess the number of phenolics in AEMK and MEMK in terms of GA equivalent gram in dry weight (GA/g dw). Different concentrations of AEMK, MEMK and GA were added to each test tube individually containing 3 ml of ethanol, 100µl of Folin-Ciocalteu reagent solution and 100µl of 100mg/ml sodium carbonate was added after 5 min. These reaction mixture tubes were kept aside for 2 h. Absorbance was measured at 765 nm.

Total flavonoids content estimation

Aluminium chloride colourimetric method was used for flavonoids determination of AEMK, MEMK and Rutin (RT) with slight modification in the method of (Zhishen *et al.,* 1999). For quantitative analysis of flavonoids, RT in the different range of concentrations was determined and a calibration curve was drawn to assess the quantity of flavonoid in AEMK and MEMK in terms of RT equivalent gram in dry weight (RT/g dw). Different concentrations of AEMK, MEMK and RT were added separately to each test tube containing 3 ml of ethanol; 100µl of 20% $AlCl_3$ in ethanol and 100µl of 5% sodium acetate. Tubes were kept incubated at room temperature for 30 min. Absorbance was measured at 415 nm.

Antioxidant *in-vitro* assay by DPPH free radical scavenging method

The capacity of AEMK, MEMK and Ascorbic Acid (AA) to scavenge the stable DPPH free radicals was measured in the method of (Duan *et al.,* 2007). DPPH radicals accept either an electron or hydrogen to form a stable diamagnetic molecule. It reacts with reducing agents then losing colour stoichiometrically with the number of electrons consumed (Gupta *et al.,* 2009); measured by spectrophotometer at 517 nm wavelength. Different concentrations of AEMK, MEMK and AA were mixed with 1ml of 0.1 mM DPPH and kept incubated in dark room at normal temperature for 30 min. After incubation, the optical density of these incubated tubes was measured at 517 nm. Control was prepared by mixing 10µl of distilled water in place of extract with 3ml of ethanol and 1ml of 0.1 mM DPPH and absorbance was determined immediately.

The percentage of scavenged DPPH of the extract was calculated using the following formula:

$$\text{Scavenged DPPH (\%)} = \frac{\text{Ac} - \text{As}}{\text{Ac}} \times 100$$

Where, Ac = absorbance of control and As = absorbance of the sample.

Antidiabetic *in vitro* assay by glucose uptake method

Various concentrations of AEMK, MEMK and Metronidazole (MTZ) were added to 1 ml of glucose solution (25 mM) and incubated together for 10 min at 37°C in the method of (Cirillo 1969). The reaction mixture was started by adding 100 µl of yeast suspension followed by vortexing and further incubation at 37°C for 60 min. After 60 min, the tubes were centrifuged and the amount of glucose was estimated in the supernatant. MTZ was used as standard drug. Absorbance was measured at 540 nm and all reaction mixture tubes were carried out in triplicates. The percentage increase in glucose uptake by yeast cells was calculated using the following formula:

$$\text{Increase in glucose uptake (\%)} = \frac{As - Ac}{As} \times 100$$

Where, Ac= the absorbance of the control reaction (containing all reagents except extract or standard) and As = absorbance of the extract or standard.

RESULTS

Percentage Yield

Percentage yield was found to be AEMK (14.35%) and MEMK (13.75%) with respect to the total quantity of powder used for the extraction.

Phytochemical investigation

AEMK and MEMK showed positive results in phenolics, flavonoids, saponins, alkaloids and glycosides in the preliminary phytochemical screening tests.

Total phenolics content estimation

Standard calibration curve of GA was estimated quantitatively in terms of Gallic Acid Equivalent gram in dry weight (GAE/g dw) and total phenolic contents of AEMK, MEMK and GA with varied concentrations were assessed (Table 1).

AEMK contains 2.214 mg GAE/g dw; MEMK contains 2.591 mg GAE/g dw

Total flavonoid contents estimation

Standard calibration curve of rutin was estimated quantitatively in terms of Rutin Equivalent gram in dry weight (RTE/g dw) and total flavonoid contents of AEMK, MEMK and RT with varied concentrations were evaluated (Table 2).

AEMK contains 2.737 mg RTE/g dw; MEMK contains 5.248 mg RTE/g dw

Table 1: Total flavonoid contents of *Murraya koenigii* leaves extract

Concentration (µL)	GA	MEMK	AEMK
100	0.163 ± 0.01	0.066 ± 0.038	0.0243 ± 0.004
200	0.314 ± 0.01	0.047 ± 0.012	0.0313 ± 0.002
300	0.433 ± 0.04	0.110 ± 0.024	0.115 ± 0.019
400	0.610 ± 0.03	0.290 ± 0.026	0.276 ± 0.054
500	0.792 ± 0.03	0.801 ± 0.325	0.431 ± 0.018

Values were expressed in Mean ± SD. Values were taken in triplicates, GA- Gallic acid, AEMK- Aqueous extract of *Murraya koenigii* leaves, MEMK - Methanolic extract of *Murraya koenigii* leaves

Table 2: Total flavonoid contents of *Murraya koenigii* leaves extract

Concentration (µL)	RT	MEMK	AEMK
100	0.019 ± 0.0005	0.011 ± 0.002	0.002 ± 0.001
200	0.039 ± 0.020	0.013 ± 0.012	0.003 ± 0.0005
300	0.044 ± 0.011	0.054 ± 0.006	0.007 ± 0.0005
400	0.053 ± 0.010	0.315 ± 0.073	0.040 ± 0.0005
500	0.052 ± 0.011	1.976 ± 0.273	0.289 ± 0.022

Values were expressed in Mean ± SD. Values were taken in triplicates, RT- Rutin, AEMK- Aqueous extract of *Murraya koenigii* leaves, MEMK -Methanolic extract of *Murraya koenigii* leaves

Antioxidant *in-vitro* assay: DPPH free radical scavenging method

DPPH free radical scavenging assay was performed to determine the antioxidant capacity of AEMK, MEMK and AA as a standard reference (Fig. 1).

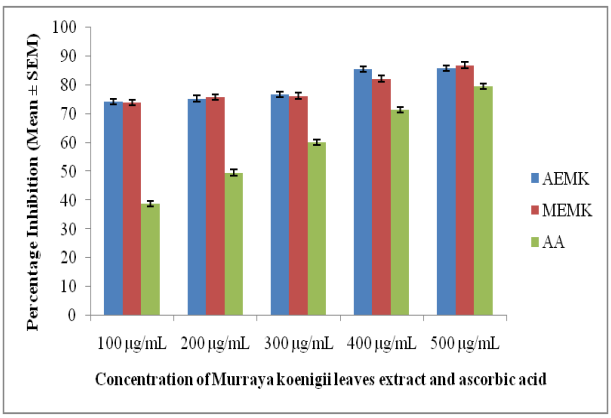

Figure 1: Antioxidant *in-vitro* DPPH assay of *Murraya koenigii* leaves extract

AA- Ascorbic acid, AEMK- Aqueous extract of *Murraya koenigii* leaves, MEMK -Methanolic extract of *Murraya koenigii* leaves

In-vitro antidiabetic assay: Glucose uptake in yeast cells method

The in-vitro antidiabetic assay was performed to determine the antidiabetic capacity of AEMK, MEMK and MTZ as a standard reference. It was observed that the glucose uptake rate is directly proportional to the concentration of the plant extract and inversely proportional to the extracellular glucose concentration (Fig. 2).

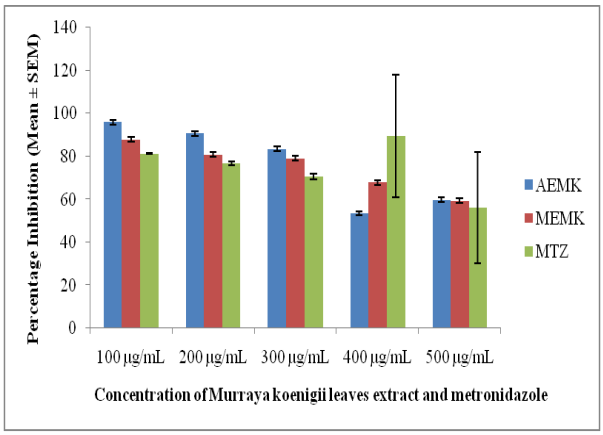

Figure 2: Antidiabetic *in-vitro* activity of *Murraya koenigii* leaves extract
AEMK- Aqueous extract of *Murraya koenigii* leaves, MEMK -Methanolic extract of *Murraya koenigii* leaves, MTZ- Metronidazole

DISCUSSION

In the preliminary phytochemical screening tests, methanolic and aqueous extracts of *Murraya koenigii* showed the presence of phenolics, flavonoids, saponins, alkaloids, and glycosides. The finding of the DPPH assay has demonstrated in the present study suggesting the free radical scavenging properties of the plant and may prevent the destruction of pancreatic cells. Our results in the study are in agreement with the antioxidant property of the plant in the study as reported by (Yadav *et al.,* 2002). Our findings of DPPH assay conform with the study by (Gupta *et al.,* 2009) that various extracts of *Murraya koenigii* leaves are quite proven as free radical scavengers. This can be attributed to flavonoids and phenols present in the extract (Gupta *et al.,* 2009). The transport of glucose across yeast cell membrane occurs by facilitated diffusion down the concentration gradient only if the intracellular glucose is effectively utilized as reported in the study by (Ahmed *et al.,* 2009). The amount of glucose remaining in the medium after a specific time interval serves as an indicator of the glucose uptake by the yeast cells in the study by (Bhutkar *et al.,* 2013). It suggests that the ability of plant for the effective glucose uptake indicates the effective glucose utilization *in-vitro*, thereby controlling blood sugar level. In our previous study, it is observed that the inhibition of glycation end products leads to the increased uptake of glucose by yeast cells (Hendre *et al.,* 2018)

CONCLUSION

Methanolic and aqueous extracts of *Murraya koenigii* Linn showed increased free radical scavenging activity with the increasing concentration of the extracts. High contents of the total phenolics and total flavonoid of the plant possess the antioxidant activity. Both extracts exhibited the highest antidiabetic activity in the glucose uptake method. Thus, curry leaves may be a good natural source of antioxidative and antidiabetic compounds to prevent oxidative damage and to reduce blood sugar level.

Acknowledgement

Authors are grateful to Hon'ble Chairman Dr. Suresh J. Bhosale and Research Fund Allotment Committee for providing financial assistance and facilities to the Ph. D research project.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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