Abrahamia
Vol. 7 (1)
11-15
2021
ISSN: 2395-4108

Anti-oxidant and anti-hyperglycemic activity of *Memecylon angustifolium* L. (Melastomaceae)

Rahul S. Raj, Vinitha S. Babu & P. M. Radhamany*
Plant Reproductive Biology Laboratory, Department of Botany,
University of Kerala, Kariavattom, Thiruvananthapuram-695 581
*Corresponding author: radhamany_m@rediffmail.com

Abstract

The present work has been done with an objective to analyze the anti-oxidant and anti-diabetic effect of leaf extract of *Memecylon angustifolium*. Fresh leaves of *M. angustifolium* were extracted with solvents based on their polarity viz, petroleum ether, chloroform, ethyl acetate, ethanol and distilled water, thereby subjected to successive soxhlet extraction. It was found that the leaf ethanolic extract possessed maximum yield and hence this was checked for the plausible anti-oxidant and anti-diabetic potential. Among the anti-oxidant assays conducted, significant results were obtained for ABTS free radical scavenging assay (IC50: 31.07±0.34) µg/mL as compared to that of DPPH assay [(IC50: 35.308±0.16) µg/mL]. A significant hypoglycemic activity was revealed by the ethanolic leaf extract [IC50: (23.14±0.06) µg/mL]. The present work suggests that the leaf ethanolic extract of *M. angustifolium* has significantly higher anti-oxidant and anti-diabetic property. Hence, this extract can help in preventing or slowing down the occurrence of diabetes, but a detailed analysis of these extracts is required to determine the presence of promising compound(s) responsible for their anti-diabetic potential.

Keywords: Anti-oxidant, Ethanolic extract, Anti-diabetic, Secondary metabolites

1. Introduction

Plants are important sources of medicine and they play a key role in world health. Medicinal herbs have been known to be an important potential source of therapeutics or curative agents. The use of medicinal plants has attained a commanding role in health system all over the world. Two-third of the world's population depends on herbal medicine for primary health care. Because of their better cultural acceptability, better compatibility and adaptability with the human body and pose lesser side effects. Medicinal plants provide a large variety of potent drugs to alleviate or eradicate infections and sufferings from diseases. Advancement in the modern medicine has alternatively increased the side effects caused by the synthetic drugs. So, the use of plant-based drugs all over world is increasing. This has brought an urgent need to develop safer drugs (Zainol et al. 2003).

Diabetes mellitus (DM) is a group of metabolic disorders characterized by a deficiency in insulin production and its action or both. Poorly controlled diabetes can lead to serious consequences causing damage to a wide range of our body's organ and tissues including heart, kidneys, eyes and nerves. Also, it leads to prolonged hyperglycemia with disturbances in most metabolic processes inside the human body. Both insulin and glucagon are hormones made by pancreas, an organ located behind stomach and are responsible for controlling blood glucose level within the body in a requisite level based on the body needs. Normally, insulin secretion is a process in the human body that primarily occurs in response to glucose levels in the blood becoming elevated. Hyperglycaemia is still considered the principal cause of diabetic complications attributed to increased production of ROS, breakdown of

starch by α-amylase, absorption of glucose by α-glucosidase and the formation of AGEs, among others. Failure of existing antidiabetic drugs due to complications and side effects are forcing researchers to look for complementary medicines for management of diabetes (Surya et al. 2013).

Memecylon angustifolium is a plant that belongs to family Melastomataceae. It is used for medicinal purposes in Asia-Pacific regions. The genus consists of more than 300 species, mainly in the Old-World tropics. Around 40 species of this genus are present now in India, out of which 21 are endemics. The major centre of diversity of Indian *Memecylon* species are present in the Western Ghats. In Ayurveda and Siddha, several *Memecylon* species are reported to be used by tribals in the treatment of skin disorders, stomach disorders, herpes, chickenpox, leucorrhoea, polyuria, menorrhagia, dysentery and also in the treatment of bacterial infections and inflammations. The leaves of *M. umbellatum* are used to treat a snake bite, it is either given orally or in the form of infusion. The fruit of *M. malabaricum* is used to control sterility in men as a part of ethnomedicine, and the juice extracted from the leaves of *M. capitellatum* is taken orally for a month to treat diabetes. Literature review suggests that the barks obtained from *M. angustifolium* is used as a tonic and refrigerant. Owing to above considerations, the present work has been conducted with an objective to determine the presence of secondary metabolites through preliminary phytochemical screening, anti-oxidant activity and anti-hyperglycemic activity in *M. angustifolium*.

2. Materials and Methods

2.1 Collection and authentication of plant material

Fresh leaves of *Memecylon angustifolium* were collected from Chellanji, Thiruvananthapuram (8.505° N; 76.947° E) and was maintained in the green house, Department of Botany, University of Kerala, Kariavattom. The botanical identities were verified by the Curator, Department of Botany, University of Kerala , taxonomical features were critically studied and confirmed with the Flora of the Presidency of Madras (Gamble 1918) ,and with other relevant available literatures. A voucher speciman was deposited at the Herbarium, Department of Botany, University of Kerala. The collected leaves were washed, shade dried at room temperature, blended in a mechanical blender, sieved and kept in airtight containers for further phytochemical analyses.

Preparation of plant extract

About 15 mg of powdered leaves of *M. angustifolium* was weighed and placed in a soxhlet apparatus and was subjected to serial extraction. The extractants, petroleum ether, chloroform, ethyl acetate, ethanol and distilled water were selected based on the polarity according to Sofowara (1993). The source extract was then placed in glass thimble of the extraction chamber (Soxhlet apparatus), which is placed on top of a distillation flask containing 150 ml of extractants. The distillation flask was placed on a heating mantle. A reflux condenser was placed atop the flask. When a certain level of condensed solvent was accumulated in the thimble, it turned colourless. The solid remains in the thimble were discarded. The extracts were filtered and concentrated separately using rotary evaporator yielding the extracted compound. The crude extract was filtered and poured in petri plate and the percentage yield was calculated using the formula;

$$\text{Percentage Yield} = \frac{\text{Weight of the product after evaporation}}{\text{Weight of the power taken}} \times 100$$

Pharmacological evaluation

The pharmacological evaluation was carried out to check the anti-oxidant and anti-diabetic potential of *M. angustifolium*.

Antioxidant activity

The samples were dissolved in methanol (95% v/v) to get 1 mg/mL concentration and utilized for antioxidant assays.

DPPH radical scavenging assay

The radical scavenging activity of leaf extracts was determined by using DPPH assay according to Chang (2019) with minor modifications. The principle of this assay is that 1, 1-diphenyl-2-picryl hydrazyl is a stable free radical with red colour which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or was measured at 517 nm. Ascorbic acid (10 mg/ mL DMSO) was used as a reference. The DPPH solution was prepared in methanol (95%) toward getting a concentration of 240 µg/mL. The stock solution of 1 mg/ mL was prepared by mixing crude extracts with 95% methanol solution. The stock solution was used for the preparation of test solution through

dilution with methanol to get the appropriate concentrations (10, 20, 30, 40 and 50 µg/mL). A standard solution of AA was prepared in the same way as described above. A freshly prepared DPPH solution (4 mL) was mixed in each of the test tubes having 100 µL extracts. The mixture was vigorously shaken and placed aside for the 30 min reaction period at room temperature in a dark room. After incubation, the absorbance of the mixture was recorded by UV spectrophotometer at 517 nm against methanol as a blank and experimental procedure was repeated for three times. The control used for the study was DPPH solution without sample solution. The percentage scavenging activity at different concentrations was determined by the following formula;

$$\text{DPPH radical scavenging activity (\%)} = ([1- (A1/A0)] \times 100)$$

where, A0 is the absorbance of control reaction, A1 is the absorbance of test compound/standard solution.

ABTS radical scavenging assay

The ABTS radical cation method was modified to evaluate the free radical scavenging effect of leaf extracts of *M. angustifolium*. The ABTS reagent was prepared by mixing 5 mL of 7 mM ABTS with 88 µL of 140 mM potassium persulfate. The mixture was then kept in the dark at room temperature for 16 h to allow free radical generation and was then diluted with water (1:44 v/v). To determine the scavenging activity, 100 µL ABTS reagent was mixed with 100 µL of each extract and was incubated at room temperature for 30 min. The stock solution was used for the preparation of test solution through dilution with methanol to get the appropriate concentrations (10, 20, 30, 40 and 50 µg/mL). A standard solution of AA was prepared in the same way as described above. After incubation, the absorbance was measured at 734 nm using a UV spectrophotometer against methanol as a blank and experimental procedure was repeated three times. The control used for the study was ABTS solution without sample solution. The ABTS scavenging effect was measured using the following formula:

$$\text{ABTS radical scavenging activity (\%)} = ([1- (A1/A0)] \times 100)$$

where, A0 is the absorbance of control reaction, A1 is the absorbance of test compound/standard solution.

Anti-diabetic Assay by alpha-amylase inhibition method

The α-amylase inhibition assay was performed using 3,5 dinitro salycylic acid (DNSA) method of Miller (1959) with slight modifications. The crude extract was dissolved in a minimum amount of 10% DMSO and was further dissolved in buffer ((Na_2HPO_4/NaH_2PO_4 (0.02 M), NaCl (0.006 M) at pH 6.9) to give concentrations ranging from 25 to 200 µg/mL. A volume of 200 µL of α-amylase solution (2 units/mL) was mixed with 200 µl each of the extracts and was incubated for 10 min at 30 °C. Thereafter, 200 µl of the starch solution (1% in water (w/v)) was added to each tube and incubated for 3 min. The reaction was terminated by the addition of 200 µL DNSA reagent (12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 mM of 3,5-dinitrosalicylic acid solution) and was boiled for 10 min in a water bath at 85–90 °C. The mixture was cooled to ambient temperature and was diluted with 5 mL of distilled water, and the absorbance was measured at 540 nm using a UV-Visible spectrophotometer. The blank with 100% enzyme activity was prepared by replacing the plant extract with 200 µL of buffer. A blank reaction was similarly prepared using plant extracts at each concentration in the absence of the enzyme solution. A positive control sample was prepared using acarbose (100 µg/mL µg/ml), and the reaction was performed similarly to the reaction with plant extract as mentioned above. The % α-amylase inhibition was plotted against the extract concentration and the IC50 values were obtained from the graph. The α-amylase inhibitory activity was calculated using the equation given below:

$$\text{Percentage of alpha amylase inhibition (\%)} = ([1- (A1/A0)] \times 100)$$

where, A0 is the absorbance of control reaction, A1 is the absorbance of test compound/standard solution.

Results and Discussion

Plants contain a myriad of natural compounds which exhibit important bioactive properties. These compounds may provide alternatives to current medications and afford a significant avenue for new drug discovery. Herbal medicines have played an important role in the health, culture and traditions of Indian people prior to the arrival of Europeans. Much of our understanding of the medicinal potential of our native plants is from accounts of Ayurvedic literature. However, traditional knowledge of plants as therapeutics is disappearing as the aboriginal culture merges into main stream society and the passing of oral traditions between each generation diminishes. Considering the diverse nature of the flora present

and the diminishing traditional knowledge, many of the Indian plants remain relatively unexplored and it is surprising that much research has not been done. Most of our understanding of Indian medicinal plants is fragmented, with the exception of some ancient literatures. Hence, the present work has been conducted with an objective to determine the anti-oxidant and anti-diabetic activity of *Memecylon angustifolium,* an unexplored species of southern Western Ghats **.**

The phytochemical extraction was carried out in different extractants like petroleum ether, chloroform, ethyl acetate, ethanol and distilled water on the basis on their polarity. The percentage yield for all the extracts along with the nature, colour and consistency were determined. It was revealed that the ethanolic extracts showed maximum yield. The results of the same were summarized in Table 1.

Table 1
Results of Yield, Colour and Consistency of Extracts

Solvent	Colour*	Consistency	Yield (%)
Petroleum Ether	Lettuce Green	Sticky	11.044
Chloroform	Ivy Green	Sticky	2.920
Ethyl acetate	Agathia Green	Sticky	2.686
Ethanol	Dahlia Purple	Non-Sticky	15.057
Water	Garnet Brown	Non-Sticky	6.441

*According to the Wilson Color Chart

The pharmacological assay was carried out in the ethanolic extract of *M. angustifolium*. Both antioxidant and anti-diabetic assays were checked in the extract. Oxidative stress is an unavoidable aspect of aerobic life. It is the result of an imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses in living organisms (Nishida, 2011). Free radicals possess the ability to induce oxidative damage associated with many diseases including neurodegenerative discases, cancer, cardiovascular disease, cataract and AIDS (Pietta et al., 1998; Middleton et al., 2000). Antioxidants, through their free radical scavenging power, are useful for the management of these discases. In addition to their role as free-radical scavengers, antioxidants also act as complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen (Andlauer and Furst,1998).

The antioxidant property of the sample was determined by the free radical scavenging activity of the sample by 2, 2-diphenyl-1-pricyl-hydroxyl radical scavenging assay (DPPH) and by ABTS radical cation decolorization assay. Ascorbic acid was used as the standard in both the assays. The 1C50 value of the tested sample was found to be higher than the standard. IC50 represents the concentration at which a substance exerts half of its maximal inhibitory effect. This value is used to characterize the effectiveness of an antagonist in inhibiting a specific biological or biochemical process. It was found that, the extract possessed a dose dependent activity which indicates that, the as the concentration of the extract increased, DPPH free radical scavenging activity as well as ABTS scavenging activity also increased, thus ensuring the efficacy of the extract to be used as an antioxidant.

Table 2
Results of DPPH Free Radical Scavenging Assay

Concentration (µg/ml)	Percentage inhibition of *M. angustifolium*	Percentage inhibition of Ascorbic Acid
10	20.975 + 0.05	55.8±0.58
20	36.824 + 0.06	62.2±0.86
30	49.91 + 0.001	75.4±0.74
40	56.27 + 0.02	77.8±0.58
50	62.12 + 0.11	82.01±0.02
IC50	35.308±0.16	22.33±0.09

*Values taken as Mean±SE

Table 3
Results of ABTS Free Radical Scavenging Assay

Concentration (µg/ml)	Percentage inhibition of *M. angustifolium*	Percentage inhibition of Ascorbic Acid
10	87.76±1.98	55.8±0.58
20	88.28±0.18	62.2±0.86
30	90.28±0.09	75.4±0.74
40	92.06±0.14	77.8±0.58
50	96.28±0.17	82.01±0.02
IC50	28.07±0.34	22.33±0.09

*Values taken as Mean±SE

Anti-diabetic assay by alpha amylase inhibition

Anti-diabetic drugs are medicines developed to stabilise and control blood glucose levels amongst people with diabetes. Drugs used in diabetes treat diabetes mellitus by altering the glucose level in the blood. With the exceptions of insulin, most GLP receptor agonists, and pramlintide, all are administered orally and are thus also called oral hypoglycemic agents or oral antihyperglycemic agents. Antidiabetic drugs (except insulin) are all pharmacological agents that have been approved for hyperglycemic treatment in type 2 diabetes mellitus (DM). If lifestyle modifications (weight loss, dietary modification, and exercise) do not sufficiently reduce HbA1c levels (target level: □ 7%), pharmacological treatment with antidiabetic drugs should be initiated. Since, *M. angustifolium* is used in traditional medicine to manage diabetes, the preliminary studies on the inhibition of α - amylase by the crude extract was determined in the present study. The extract has shown dose-dependent activity and a maximum of 72.20% of inhibition at 200 µg/ml. The percent inhibition has shown a steady increase and was dose dependent. IC50 value of the ethanolic extract (23.14±0.006) was comparable to the standard acarbose (18.12±0.005). The results of the same were depicted in Table 4.

Table 4
Results of alpha amylase inhibition assay

Concentration (µg/ml)	Percentage inhibition of *M. angustifolium*	Percentage inhibition of Acarbose
25	52.4±0.50	67.0±0.70
50	67.8±0.58	76.0±0.70
100	76.8±0.86	82.4±0.50
200	72.2±0.58	77.6±0.50
IC50	23.14±0.06	18.12±0.005

*Values taken as Mean±SE

Conclusion

A balance between free radicals and antioxidants is necessary for proper physiological function. If free radicals overwhelm the body's ability to regulate them, a condition known as oxidative stress ensues. Free radicals thus adversely alter lipids, proteins, and DNA and trigger a number of human diseases, of which diabetes mellitus is a serious concern. According to ancient literatures the genus *Memecylon* has good antidiabetic property. Till date, there has been no report of the anti-hyperglycemic activity in *M. angustifolium*. In the present work, only the crude extract of the plant material was used so that it is too tough to determine the compound behind the activity. Thus, for further confirmation a detailed analysis of the extract is necessary.

Acknowledgement

The authors thank Head, Department of Botany, University of Kerala for providing necessary facilities for the conduct of the present work.

References

Andlauer, W., Furst, P., (1998). Antioxidative power of phytochemicals with special reference to cereals. Cereal Foods World. Volume 43 Page no: 356-359.

Chang, C., Meikle, T. G., Su, Y., Wang, X., Dekiwadia, C., Drummond, C. J., Yang, Y. (2019). Encapsulation in egg white protein nanoparticles protects anti-oxidant activity of curcumin. Food chemistry, 280, 65-72.

Gamble, J. S. (1920). The Flora of Madras: III. Bulletin of Miscellaneous Information (Royal Botanic Gardens, Kew), 49-57.

Middleton. E Jr., Kandaswami, C., Theoharides, T, C., (2000). The effects of plant Flavonoids on mammalian cells: implications for inflammation, Heart-disease, and cancer. Volume 52 Issue 4 Page no: 673-75.

Miller, G, L., (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem, 31: 426–428.

Nishida, H., Hamamoto, M., Sugiyama, J., (2011). Drafting Genome sequencing of the yeast *Saitoella complicata*. J Gen Appl Microbiol. Volume 57, Issue 4, Page no: 243-246.

Pietta, P., Simonetti, P., Mauri, P., (1998). Antioxidant Activity of Selected Medicinal Plants. Journal Agric. Food Chem. Volume 46 Page no: 4487-4490.

Sofowara, A. (1993). Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria. p. 289.

Surendran Surya, Abdul Dhaliya Salam, Dawn Vallikattukuzhiyil Tomy, Betty Carla, Ravindrakurup Arun Kumar, Christudas Sunil (2013), Diabetes mellitus and medicinal plants-a review; Asian Pacific Journal of Tropical Disease 4 (5), 337-347, 2014.

Zainol MK, Abd Hamid A, Yusuf S, Muse R, (2003). Anti-oxidative activity and total Phenolic Compounds of leaf, root and petiole of four Accessions of *Centella asiatica* Urban. Food Chem. Volume 81, Page no: 575-81.

Received: 12 March 2021
Revised & Accepted: 30 April 2021