IJPSR (2019), Volume 10, Issue 6 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 26 September 2018; received in revised form, 02 January 2019; accepted, 11 January 2019; published 01 June 2019

ANTIOXIDANT AND ANTI-DIABETIC POTENTIAL OF *OPERCULINA TURPETHUM* (L.) SILVA MANSO LEAF

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Keywords:

Operculina, Anti-diabetic, Flavonoids, Ethyl acetate fraction

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ABSTRACT: 'Natural curing agents for diabetes' is a matter of world research. Diabetes, the gift of altered life pattern, affected considerably larger proportion of world population. Ancient Indian literature has the treasure of knowledge regarding natural curing agents. The plant *Operculina turpethum* (Convolvulaceae) is one among them. The plant root is used in many Ayurvedic formulations to treat various diseases including diabetes. In the present study, anti-oxidant and anti-diabetic properties of leaf extract were done to find an alternative for root with an aim to promote sustainable utilization of the plant in future. The methanol extract of the leaf was fractionated with hexane and ethyl acetate, and the residue was dissolved in methanol. The antioxidant and hypoglycemic effect of these fractions were analyzed using standard protocols. The results projected anti-diabetic efficacy of the plant leaf and also powerful anti-diabetic agents in ethyl acetate fraction. The phytochemical quantification of the flavonoid content in the fractions revealed considerably high amount of flavonoids in ethyl acetate fraction than the other two. Based on this preliminary results, it is suggested that *O. thurpetum* is a promising plant for future development of anti-diabetic drug.

INTRODUCTION: Reactive oxygen species and reactive nitrogen species are two groups of free radicals that initiate a series of chemical changes such as lipid peroxidation and enzyme denaturation in living organisms and thereby damage cell structures and interrupt metabolic pathways. The reactions of free radicals in bodies lead to cancers, neurodegenerative diseases, liver disorders, cardiac disorders, diabetes, and skin disorders.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(6).2898-01

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(6).2898-01

Even though free radicals have a role in the host's defense mechanism, higher concentrations of free radicals in living systems produce deleterious effects. The balancing of such free radicals is generally achieved through the activity of antioxidant compounds synthesized in the body (endogenous antioxidants) or reaching the body through the diet (dietary antioxidants).

Antioxidants terminate the chain reaction initiated by the free radicals and thereby prevent the damage of body parts. Plants harbor various types of antioxidants such as vitamin C, vitamin E, β-carotene, lycopene, flavonoids, phenolic acids and various minerals [1]. Diabetes mellitus is a very serious health issue that shows an alarming increase in the worldwide occurrence.

Global Diabetes Report says that more than 422 million in the world are suffering from diabetes [2]. As diabetes leads to several complications such as blindness, kidney failure, stroke, lower limb amputation and heart attacks, it is regarded as a serious health issue. Many of the anti-diabetic drugs such as Pioglitazone were reported to have side effects. So, search for natural agents to treat diabetes have given worldwide attention on these days. Several studies bring about the plant-derived anti-diabetic products like diasulin, pancreatic tonic 180 cp, chakrapani, diabecon, bitter gourd powder, dia-car, diabetes-daily care, gurmar powder, epinsulin, diabecure, syndrex and diabeta. These are now sold as anti-diabetic agents after successful clinical studies [3]. Ancient literature mentioned anti-diabetic effects of *Operculina turpethum* root. Beta-sitosterol, a phytosterol identified from the root of the plant is reported to have anti-diabetic effect [4].

The main objective of the present work is to identify the bioactive efficiency of *Operculina turpethum* leaf.

MATERIALS AND METHODS: Leaves harvested from mature plants grown in the Botanic garden, Department of Botany, University of Kerala (Voucher specimen number: KUBH 6078) during 2015 were used for the analysis. The shade dried leaves were powdered with the help of a blender. Then 100 g fine leaf powder was extracted with methanol. The concentrated methanol extract was further fractionated by using hexane and ethyl acetate, and the residue was dissolved in methanol. These fractions were used for further studies.

Quantification of Flavonoids: The fractions were subjected to quantification of flavonoids as per the aluminum chloride method [5]. The concentration of the flavonoid in the test samples were estimated from the standard graph prepared with the standard quercetin, and the result was expressed as mg equivalent of quercetin/g of sample.

***In-vitro* Antioxidant Assays:** The free radical scavenging efficiency of fractions was determined from DPPH, hydroxyl and nitric oxide - free radical scavenging assays and also by measuring their reducing power. All experiments were performed in triplicate, and the percentage inhibition was calculated.

According to the modified method of Chew *et al*. (2008) [6], the DPPH free radical scavenging efficiency of four different concentrations (50, 100, 200, 400 and 800 µg/ml) of the three fractions was measured. The color intensity of the mixture after incubation was recorded at 517 nm against the reagent blank. The scavenging activity of reference compound ascorbic acid was also measured. Deoxyribose method was employed to analyze the hydroxyl radical scavenging activity [7]. The color intensity developed in 50, 100, 200, 400 and 800 µg/ml of the hexane, ethyl acetate and methanol fractions were measured at 532 nm against the reagent blank. Gallic acid was used as the standard.

Spectrophotometric determination of nitric oxide scavenging activity was done according to the method of Rice- Evans *et al*., (1996) [8]. Four different concentrations (50, 100, 200, 400 and 800 µg/ml) of the hexane, ethyl acetate and methanol fractions and control were screened for their ability to scavenge nitric oxide free radical. The absorbance of the chromophore formed during diazotization of the nitrate with sulphanilamide and subsequent coupling with N-1 naphthyl ethylenediamine dihydrochloride was measured at 546 nm, and the percentage scavenging activity was measured concerning the standard Gallic acid.

The percentage antioxidant activity in each of the above assays was calculated by using the standard formula:

$$\% \text{ inhibition} = [(A_0-A_1)/A_0] \times 100$$

Where, A_0 is the absorbance of the control, and A_1 is the absorbance of the sample in the presence of extract after the reaction has taken place. The EC_{50} values were calculated, and the result was compared with that of the standards.

Determination of Reducing Power: Reducing power was determined according to the method of Oyaizu (1986) [9]. Different concentrations (50, 100, 200, 400 and 800 µg/ml) of the hexane, ethyl acetate, and methanol fractions were mixed with reagents as prescribed in the methodology. The solutions were observed for the formation of Pearl's Prussian blue color and the intensity of color was measured at 700 nm. The increase in absorbance denotes the increase in reducing the power of the fractions.

***In-vitro* Hypoglycemic Activity:** The α-amylase inhibitory activity of the three fractions was determined spectrophotometrically according to the method of Kumar *et al*., (2011) [10] to monitor the hypoglycemic effects. Five concentrations (12.5, 25, 50, 75 and 100 µg/ml) of fractions and the positive control rutin was prepared. To 0.5 ml of sample and 0.5 mg/ml of α-amylase solution was added and incubated at 25 °C for 30 min. Then, 0.5 ml of starch solution was added to the reaction mixture and incubated at 25 °C for 20 min and the reaction was stopped using 1ml of dinitro salicylic acid (DNSA). The mixture was then incubated at 90 °C for 5 min and cooled to room temperature. The reaction mixture was diluted by the addition of 10 ml distilled water and absorbance was measured at 540 nm against a reagent blank (negative control). α-Amylase inhibition activity was calculated as percentage inhibition of the enzyme α-amylase. The assay was repeated thrice to check the reproducibility of the result.

RESULTS: The bioactive efficacy of *Operculina turpethum* leaf was assessed from the results of the antioxidant activity, reducing power and hypoglycemic effect. The ethyl acetate fraction exhibited higher flavonoid concentration, reducing power and hypoglycemic effect. The flavonoid concentrations of each fraction are presented in **Table 1**. Presence of potential antioxidant compounds is suggested in the fractions from their lower EC_{50} values. The results of antioxidant activity are summarized in **Fig. 1** and reducing power of fractions are shown in **Table 2**. **Table 3** and **Fig. 2** shows the hypoglycemic effect of the fractions studied.

TABLE 1: QUANTIFICATION OF FLAVONOIDS

Fraction	Total flavonoids (mg equivalents of quercetin)
Hexane	0.18 ± 0.38
Ethyl acetate	0.40 ± 0.17
Methanol	0.22 ± 0.92

TABLE 2: REDUCING POWER OF FRACTIONS

Fraction	Concentration in µg/ml			
	100	200	300	400
Hexane	0.194 ± 0.3	0.427 ± 0.1	0.452 ± 0.7	0.572 ± 0.5
Ethyl acetate	0.805 ± 1.1	0.834 ± 1.4	0.144 ± 0.3	0.068 ± 0.4
Methanol	0.032 ± 0.7	0.052 ± 1.6	0.172 ± 1.2	0.195 ± 0.5
Ascorbic acid	0.798 ± 0.2	0.821 ± 0.6	0.884 ± 0.1	0.134 ± 0.2

TABLE 3: A-AMYLASE INHIBITION ACTIVITY OF FRACTIONS (% INHIBITION)

Fraction (µg/ml)	12.5	25	50	75	100
Hexane	38.20 ± 0.05	40.24 ± 0.24	44.88 ± 0.09	47.56 ± 0.11	52.70 ± 0.71
Ethyl acetate	39.12 ± 0.04	42.70 ± 0.07	45.17 ± 0.47	49.27 ± 0.05	54.78 ± 0.34
Methanol	36.76 ± 0.91	39.87 ± 0.07	41.23 ± 0.84	47.37 ± 0.82	50.04 ± 0.49
Rutin	16.04 ± 1.01	22.06 ± 0.51	30.65 ± 0.23	38.68 ± 0.06	45.78 ± 0.38

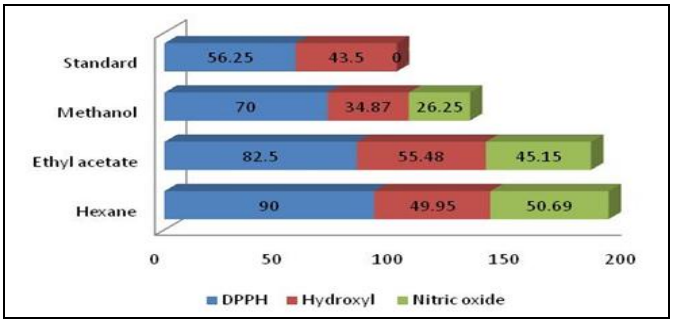

FIG. 1: EFFECTIVE CONCENTRATION OF FRACTIONS (EC_{50}, µg/ml) IN ANTIOXIDANT ACTIVITY

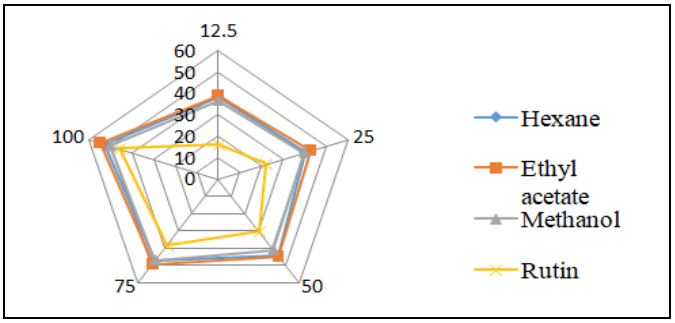

FIG. 2: A-AMYLASE INHIBITING POTENTIAL OF FRACTIONS

DISCUSSION: Pharmacology utilizes *in-vitro* assays for the preliminary evaluation of the therapeutic potential of plant extracts. The methanol extract was partitioned for the ease of study, and a relatively non-polar to polar (hexane-ethyl acetate-methanol) combination of solvents was selected for successive fractionation of crude leaf extract. Previous studies recommended liquid fractionation technique for elution of flavonoids and ethyl acetate is suggested as good solvent for flavonoid oligomers [11]. Phytochemical quantification done in the present study also

justifies the previous observations. The highest reducing power exhibited by ethyl acetate fraction may thus be correlated with the presence of flavonoids in it. While the results of other antioxidant assays suggested the synergistic action of different phytochemicals present in the fractions. An important starch-digesting enzyme α-Amylase is involved in the conversion of oligomeric carbohydrates in the diet into disaccharides such as maltose and other simpler carbohydrates.

Further digestion of disaccharides leads to the production of glucose units in the body. Through the inhibition of α-amylase activity, production and absorption of glucose can be prevented. Inhibition of α-amylase can thus be used as an effective strategy to control postprandial hyperglycemia (a condition with elevated glucose levels after a heavy meal). If not properly controlled postprandial hyperglycemia will lead to serious cardiovascular problems and other complications. Many phytochemicals such as flavonoids and tannins are believed to be the strong inhibitors of α-amylase activity and are called starch blockers [12]. These chemicals can thus produce a hypoglycemic effect in diabetic conditions.

In many previous studies, ethyl acetate fraction of plant extracts showed good hypoglycemic potential [13]. In the present study, the flavonoid-rich ethyl acetate fraction produced good inhibition of α-Amylase enzyme and thereby revealed to be a source of hypoglycemic agent/s. The hypoglycemic potential of the leaf was found to be good in comparison with the standard compound used.

CONCLUSION: As the leaf was found to be a good source of antioxidants, reducing agents and hypoglycemic agents, the study pointed out the pharmacological importance of the plant in diabetes treatment. Detailed studies with ethyl acetate fraction are required for finding the anti-diabetic compound/s from the plant.

ACKNOWLEDGEMENT: We express our sincere gratitude to Kerala State Council for Science, Technology and Environment (KSCSTE) for providing financial support for the work and the University of Kerala for the facilities to carry out the research.

CONFLICT OF INTEREST: We do not have any conflict of interest.

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How to cite this article:
Jalaj AV and Radhamany PM: Antioxidant and anti-diabetic potential of *Operculina turpethum* (L.) silva manso leaf. Int J Pharm Sci & Res 2019; 10(6): 2898-01. doi: 10.13040/IJPSR.0975-8232.10(6).2898-01.