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Original Article

Evaluation of Antioxidant and Antidiabetic Activity of Menthaarvensis Linn.

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ABSTRACT

The anti-diabetic activity of ethanol (70%) extract of *Menthaarvensis* was performed on *in vivo* model along with antioxidant activity and determination of total polyphenol content. Ethanol extract of *Menthaarvensis* contains 4.7% of total extractable polyphenols. The antioxidant activity studied on ethanol extract showed very promising result in superoxide radical, hydroxy radical and DPPH radical methods. The antioxidant activity is directly correlated to the anti-diabetic activity of drug. The in vivo anti-diabetic activity of ethanol extract of *Menthaarvensis* was performed on streptozotocin-induced diabetes mellitus showed significant inhibition of blood glucose level as compared to control and similar to that of standard glibenclamide. The overall data potentiates the traditional value of *Menthaarvensis* as an anti-diabetic drug.

Key words: Anti-diabetic, polyphenol, antioxidant, *Menthaarvensi*

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INTRODUCTION

Plants always play an important role in mankind life. Human beingsuse plants for their daily basic requirements like feeding, clothing, sheltering, hunting and nursing. As source of medicines, plants have formed the basis for sophisticated traditional systems and continue providing mankind with new remedies in treating different ailments.*M. arvensis* is a branched, strongly aromatic herb that stems up to 40 cm long with ascending terminal branches. The characteristics of Leaves are elliptic to oblong-ovate, 1.5 to 2 cm long, short-petioled, toothed margins, rounded or blunt tipped. Hairy purplish to light blue axillary flowers [1]. The plant is used to treat liver and spleen diseases, asthma and jaundice. The yield of oil is 5% by distillation of leaves which contain 40 to 50% menthol. The oil is used as antiseptic, carminative, refrigerant, stimulant and diuretic. Menthol, the main active constituent of *M. arvensis* is used in medicine for stomach disorders and in ointments for headache. The infusion of leaves is used for treatment of indigestion and rheumatic pains [2].

Diabetes mellitus is currently anincreasing global health concern. The incidence and prevalence of diabetes are growing, especially in developing and newly industrialized countries. Majority of all cases of diabetes in developed and developing countries are non- insulin-dependent diabetes mellitus, also known as type-2 diabetes (T2D), or adult-onset diabetes. These diagnoses are typically in adults more than 30 years of age[3], and are usually characterized by postprandial hyperglycemia, an abnormal rise in blood sugar following a meal[4]. Several factors integral to the T2D disease process are such as insulin resistance, hyperinsulinemia, impaired insulin secretion, reduced insulin-mediated glucose uptake and utilization[5]. Many efforts have been made to search for other effective and safe medicines from natural materials in order to control diabetes[6].

Indian traditional medicines have a long history to treat DM by the herbs and herbal extracts. *Menthaarvensi* is one of the traditional antidiabetic herbs. So far, the anti-diabetic activity of ethanol (70%) extract of *Menthaarvensis*(MAEE) was not performed on *in vivo* model. This study aimed to brief the antidiabetic properties of *Menthaarvensi* by correlating its antioxidant properties with *in vitro* and *in vivo* management of diabetes [7].

MATERIALS AND METHODS

Plant material and preparation of extract: Leaves of *M. arvensis*plant were collected from Seshachalam hills and authenticated by Dr. K. MadhavaChetty, Assistant Professor in Department of Botany, Sri Venkateshwara University,Tirupati, Chittoor district, Andhra Pradesh. The crude plant material was dried under shade and powdered mechanically to coarse powder. The coarsely powdered plant material (500g) was subjected to extraction with 70% ethanol using soxhlet extraction. The extracts were concentrated to dry residue by distillation (temperature 60 °C without vacuum) and dried completely in desiccators and weighed. The yield of the ethanol extract was 4.6 g.

Phytochemical Screening: Standard screening tests[08] were employed in screening the extracts for different constituents. Conventional protocol for detecting the presence of alkaloids, tannins, flavonoids and steroids, etc. was utilized. The ethanol extract on TLC over silica gel has showed eight distinct spots, whereas hexane, ethyl acetate and hydroalcohol extracts of each has showed four to five spots. All the extracts were subjected to determination of total polyphenol content.

Animals: Healthy adult Wistar Albino rats of 180-250 g of were selected for studying the anti-diabetic activity. The animals were obtained from Gentox laboratories, Hyderabad. The animals were housed according to CPCSEA guidelines (under standard temperature condition). They were given a pellet diet and water *ad libitum*. The ethical clearance was obtained from Institutional Animal Ethics Committee (IAEC) before the experiment.

Determination of total phenolic content in different extracts:

Preparation of standard: The total phenolic content in the plant extracts was determined by using Folin-Ciocalteucolourimetric method based on oxidation-reduction reaction [09]. Various concentrations of gallic acid solutions in methanol (10, 25, 50 and 75 µg/ml) were prepared. In a 20 ml test tube, 1 ml gallic acid of each concentration was added and to that 5 mL of Folin-Ciocalteu reagent (10%) and 4 ml of 7% Na_2CO_3 were added to get a total volume of 10 ml. The blue coloured mixture was shaken well and incubated for 30 minutes at 40 ^oC in a water bath and the phenols were determined by spectrophotometric method. The absorbance was measured at 765 nm against blank. All the experiments were carried out in triplicate and the mean value of absorbance was obtained. The average absorbance values obtained at different concentrations of gallic acid were used to plot the standard curve. The total phenolic content was expressed in terms of gallic acid equivalent (mg GA/g of extract), which is a common reference compound.

Preparation of sample: Various concentrations of the extracts (25, 50, 100 and 200 µg/mL) were prepared. Following the procedure described for

standard, absorbance for each concentration of extract was recorded. Total phenolics content of the extracts was expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g). The total phenolic content in all samples was calculated using the formula: C = c V/m where, C = total phenolic content mg GAE/g dry extract, c = concentration of gallic acid obtained from calibration curve in mg/mL, V = volume of extract in ml, m = mass of extract in gram.

Statistical analysis: All the experiments were carried out in triplicates and data reported are mean ± standard deviation. Calculation of linear correlation coefficient and correlation analysis were carried out using MS Office Excel 2007. The linear regression equation for a straight line is, Y = mx + c where, Y = absorbance of extract, m = slope of the calibration curve, x = concentration of extract, c = intercept. Using this regression equation, concentrations of extracts were calculated. From the calculated values of concentration of each extract, the total phenolics content was calculated.

Drugs: 1, 1- diphenyl-2-picrylhydrazyl (Sigma Chemical Company, St. Louis, USA), Riboflavin (LobaChemiePvt Ltd., Bombay), Deoxyribose (Sisco Research Laboratories Pvt Ltd., Mumbai), Nitrobluetetrozolium (Sisco Research Laboratories Pvt Ltd., Mumbai), All other chemicals and reagents used were of analytical grade, Ascorbic acid was utilized as reference antioxidant drug.

Determination of Superoxide Radical Scavenging Activity: Superoxide anion scavenging activity of ethanol extract of *M.arvensis* was done based on the method described by Liu, Ooi, and Hang [10] with slight modification. One millilitre of nitroblue tetrazolium (NBT) solution (156 mmol/L NBT in 100 mmol/L phosphate buffer, pH 7.4), 1ml NADH solution (468 mmol/L in 100 mmol/L phosphate buffer, pH 7.4) and 0.1 ml of sample solution of different concentrations of ET extracts in water were mixed. The reaction started by adding 100 ml of phenazinemethosulphate (PMS) solution (60 mmol/L PMS in 100 mmol/L phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25°C for 5 min, and the absorbance at 560 nm was measured against blank samples. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following

Formula: Inhibitory ratio = $[(A_0-A_1)/A_0] \times 100$

Where A_0 was the absorbance of the control, and A_1 was the absorbance of the ET extracts and standards [11].

Determination of Hydroxyl Radical Scavenging Activity: Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the ethanol extract of *M.arvensis* for hydroxyl radicals generated from the $Fe^{2+}/EDTA/H_2O_2$ system (Fenton reaction). The hydroxyl radical attacks deoxyribose, which eventually results in the formation of thiobarbituricacid reacting substances (TBARS) [12]. Fenton reaction mixture consisting of 200 µl of 10 mM ferrous sulphate ($FeSO_4$. $7H_2O$), 200 µl of 10 mM EDTA and 200 µl of 10 mM 2-deoxyribose and was mixed with 1.2 ml of 0.1 M phosphate buffer (pH 7.4) and 200 µl of plant extract. Thereafter, 200 µl of 10 mM H_2O_2 was added before the incubation at 37 ^oC for 4 h. Then, 1 ml of this Fenton reaction mixture was treated with 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 0.8% thiobarbituric acid and 1.5 ml of 20 % acetic acid. The total volume was then made to 5 ml by adding distilled water and kept in an oil bath at 100 ^oC for 1 hour. After the mixture had been cooled, 5 ml of 15:1 v/v butanol-pyridine mixture was added. Following vigorous shaking, the tubes were centrifuged at 4000 rpm for 10 min and the absorbance of the organic layer containing the thiobarbituric acid reactive substances was measured at 532 nm. A control was prepared using 0.1 ml of vehicle in the place of plant extract/ascorbic acid. The percentage inhibition of hydroxyl radicals by the extract/compound was determined by comparing the absorbance values of the control and the experimental tubes as calculated for hydroxyl radical assay.

The percentage inhibitions of superoxide production by the extracts were calculated using the formula: Inhibitory ratio = $[(A_0-A_1)/A_0] \times 100$ Where, A_0 is the absorbance of control; A_1 is the absorbance with addition of plant extract/ ascorbic acid.

Determination of 1, 1- Diphenyl-2-Picrylhydrazyl (DPPH) Radical Scavenging Activity: The free radial scavenging activity of ethanol extract of *M.arvensis*was measured in terms of hydrogen donating ability using DPPH radical as described by the method [13] with a slight modification. Briefly, 40 μl of sample extracts of different concentrations (0.05–2 mg/ml) were mixed with 200 μl of 50 μM DPPH solution in ethanol. The mixture was immediately shaken and incubated for 15 min in the dark at room temperature. The decrease in absorbance was measured at 517 nm with a microplate reader (Tecan Sunrise, Austria). Ascorbic acid (5–80

μg/ml) was used as a standard and the control was ethanol. The percentage of inhibition activity of the extracts was calculated according to the following equation:

Percent (%) inhibition of DPPH• activity = $[(A_0-A_1)/A_0] \times 100$
Where, A_0 is the absorbance of control; A_1 is the absorbance with addition of plant extract/ ascorbic acid.

The concentration of extracts required to scavenge 50% of DPPH radical was estimated from the graph plotted against the percentage inhibition and compared with the standard. All the tests were performed in triplicate, and the results were expressed as μg/ml.

Calculation of 50% inhibition concentration: The optical density obtained with each concentration of the extract/ ascorbic acid was plotted taking concentration on X-axis and percentage inhibition on Y-axis. The graphs were extrapolated to find the 50% inhibition concentrations of extracts/ ascorbic acid.

Anti-diabetic activity: The whole study was divided into five groups, each group contain six animals. Streptozotocin (60 mg/kg) was injected to rats in each group of animals following 18 h fast. After 48 h the blood glucose level of rats were determined by glucose oxidase method. Only those animals which showed hyperglycaemia (blood glucose levels more than 200 mg/dl), were considered diabetic and taken for further experimentation. The group I received vehicle only (Tween‑80, 1%) and served as control. Animals of group II, group III and group IV received MAEE 100, 200 and 400 mg/kg body wt therapeutic doses, respectively and group V received glibenclamide 1.0 mg/kg. The study was carried out after repeated (once a day) administration of the extract for 21 consecutive days. Basal glycaemia was determined in overnight fasted animals. The vehicle (1% tween 80), MAEE (100, 200 and 400 mg/kg) and glibenclamide (1.0 mg/ kg) were orally administered to the animals of group I‑V for 21 consecutive days. Blood samples collected on the 7th, 14th and 21st days were analyzed for the determination of blood glucose level[14].

Statistical Analysis: Results were analyzed using one way ANOVA method and expressed as Mean ± SEM. The statistical significance considered was $P < 0.05$ (confidence limit: 95%).

RESULTS AND DISCUSSION

In the present study we have studied phytochemical screening, total phenolic content and *in vitro* anti-oxidant and anti-diabetic activity of ethanol extract of *M.arvensis*leaves. Phytochemical screening revealed the presence of tannins, flavonoids and triterpenoids.The total phenolic content of ethanoextractis379.36 ± 6.41mg/g GAE. Total phenolic content of the extracts was calculated from the regression equation of calibration curve ($y = 0.014x$; $R^2 = 0.999$) and expressed as mg gallic acid equivalents (GE) per gram of sample in dry weight (mg/g).

Superoxide anion plays an important role in the formation of more reactive species such as hydrogen peroxide, hydroxyl radical and singlet oxygen, which induce oxidative damage in lipids, proteins and DNA [15]. Therefore, studying the scavenging activity of plant extracts/compounds on superoxide radical is one of the most important ways of clarifying the mechanism of antioxidant activity. In the present study, ethanol extract of *M.arvensis*was found to possess concentration dependent scavenging activity on superoxide generated by photoreduction of riboflavin and the results were graphically shown in Figure 1.

The mean 50% inhibition concentration (IC_{50})values for superoxide radical of ethanol extract of *M.arvensis*was found to be 147.25 µg. The mean IC_{50} value of ascorbic acid was found to be 52.5 µg. The results were given in Table 1 and Figure 4.

The ethanol extract of *M.arvensis*was found to possess concentration dependent scavenging activity on hydroxyl radicals and the results were graphically shown in Fig 2. The mean IC_{50} values for hydroxyl radical of ethanol extract of *M.arvensis*wasfound 181.22 µg. The mean IC_{50} value of ascorbic acid was found to be 66.8 µg. The results were shown in Table 1 and Figure 4.

The ethanol extract of *M.arvensis*wasfound to possess concentration dependent scavenging activity on DPPH radicals and the results were graphically shown in Fig 3. The mean IC_{50} values for DPPH radical of ethanol extract of *M.arvensis*wasfound to be 116.42 µg. The mean IC_{50} value of ascorbic acid was found to be 19.5 µg. The results were given in Table 1 and Figure 4.

Antioxidants plays a vital role in the prevention of human diseases. Herbal extracts with antioxidants activity may work as free radical scavengers, reducing agents and quenchers of single oxygen formation or reactive oxygen species, thereby protecting the health from degenerative disease such as cancer. The reactive oxygen species are damaging byproducts generated during normal cellular metabolism or from toxic injury. They lead to cause oxidative stress that contributes to the

development of number of human diseases by damaging lipids, proteins and DNA. Phenolic content have shown a good correlation with antioxidant activity, this may be due to structural differences. Phenolic compounds, such as tannins, flavonoidsand phenolic acids possess anti-inflammatory, anti-carcinogenic, anti-atherosclerotic and other properties that may be related to their antioxidant activities [16].

The ethanol extractof *M.arvensis* produced concentration dependent percentage inhibition on superoxide, hydroxyl radicalsand DPPH radicals and produced maximum activity at a concentration of 160 µg and there after the percentage inhibitions were raised gradually to its maximum level with higher concentrations.

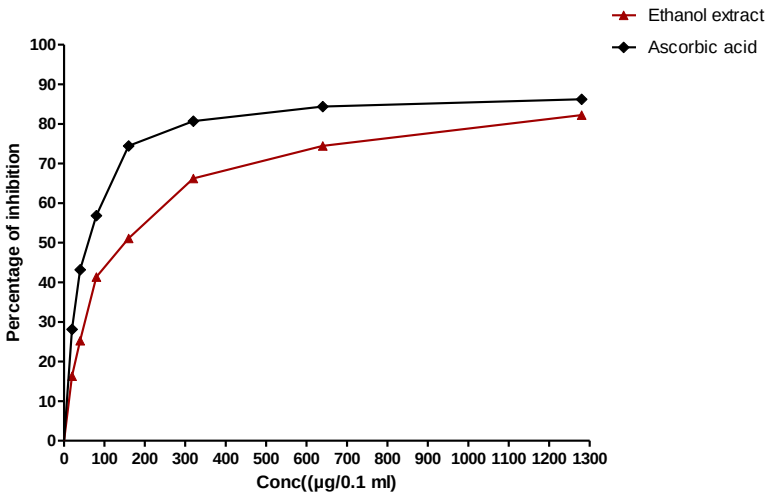

Fig 1: Concentration dependent percentage inhibition of Superoxide radical by ethanol extract of *M.arvensis*.

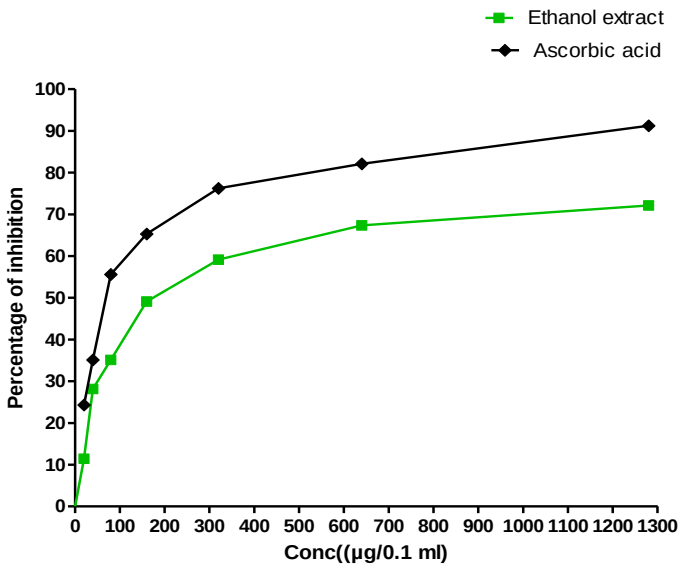

Fig 2: Concentration dependent percentage inhibition of Hydroxyl radical by ethanol extractof *M.arvensis*

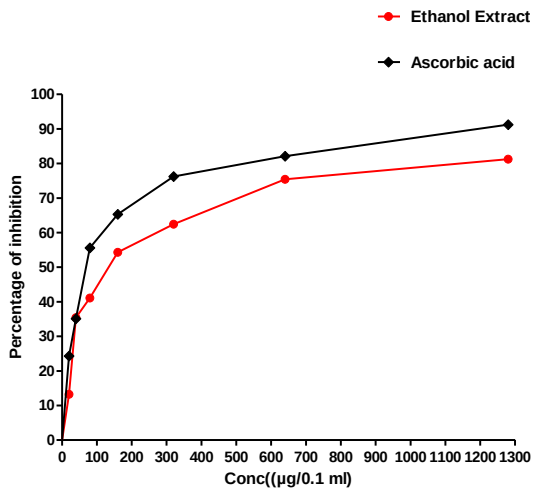

Fig 3: Concentration dependent percentage inhibition of DPPH radical by different extracts of *M.arvensis*.

Table 1: 50% Inhibition concentrations (IC_{50}) of different extracts of *M.arvensis*against Superoxide, Hydroxyl and DPPH radicals

Extracts/Compound		50% Inhibition Conc (IC_{50})		
		Superoxide radical	Hydroxyl radical	DPPH radical
M.arvensis	EE	146.25±1.3	181.22±3.1	116.42±3.2
Ascorbic acid		52.5±2.1	66.8±2.3	19.5±1.5

EAE=Ethyl acetate extract; HAE= Hydroalcoholic extract; EE=Ethyl acetate extract;HE= Hexane extract.

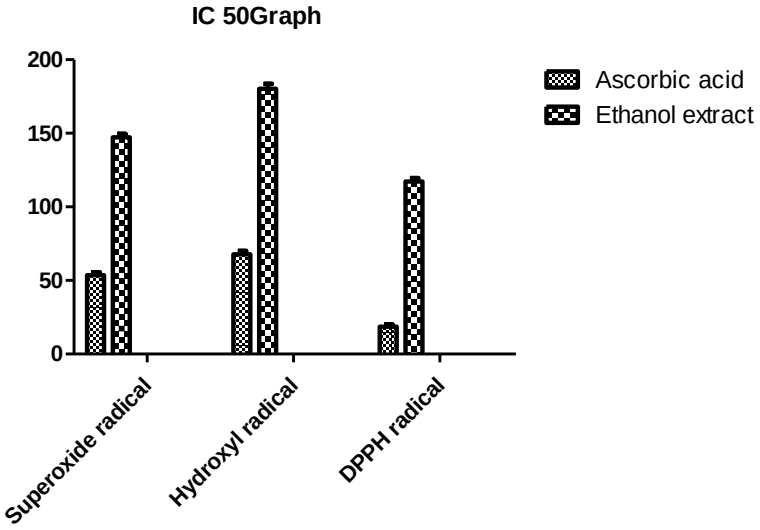

Figure 4: 50% Inhibition concentrations (IC_{50}) of different extracts of *M.arvensis*against Superoxide, Hydroxyl and DPPH radicals.

In vivo study of MAEE was done on streptozotocin induced diabetic rats. The effect of the treatment with extract and glibenclamide on blood glucose level in diabetic rats after post-treatment days was shown in Table 2. The study was conducted by collecting blood on 7th, 14th and 21st days after induction of diabetes and analyzed for the determination of blood glucose level. All the values were found significant in all the treatment doses (100, 200 and 400 mg/kg body wt) as compared to control and standard glibenclamide (Table 2). These findings suggested that ethanol extract of *M.arvensis* has anti-diabetic activity, which validatesits traditional uses in India.

Table2:Effect of repeated oral administration of extracts and glibenclamide on blood glucose level instreptozoto cin diabetic rats.

Treatment	Blood glucose profile (mg/dl)			
	0	7	14	21
Control (vehicle)	342.20±15.01	367.00±24.31	395.00±24.31	419.00±24.44
MPEE 100 mg/kg	337.05±11.53	321.05±10.74	307.05±11.53	291.05±11.53
MPEE 200 mg/kg	321.00±21.46	272.80±13.60*	244.80±16.60*	209.40±13.55*
MPEE 400 mg/kg	319.00±16.06	245.80±16.60*	219.80±16.60*	181.40±13.55*
Glibenclamide 1 mg/kg	291.00±9.44	221.00±17.84*	184.70±15.84*	164.00±11.79*

The values represent in±SEM, n=6, *<0.05. MAEE=Ethanol extract of *Mentha arvensis.*

All the extracts of *M.arvensis* roots in this research exhibited different extent of antioxidant activity in different assays. It is evident from the present study that the ethanolic extract of *M.arvensis*leaves could be used as good source of natural antioxidants in controlling *Diabetes millitus*in pharmaceutical industry.

CONCLUSION

The phytochemical screening tests indicated the presence of tannins, flavonoids and triterpenoids in the ethanol extract of *M.arvensis.* Several of such compounds are known to possess potent antioxidant and anti-diabetic activities [17]. Hence, the observed antioxidant and anti-diabetic activities may be due to the presence of any of these constituents. Further purification of these bioactive constituents may result in the development of potent antioxidant agent with low toxicity and better anti-diabetic index.

CONFLICT OF INTEREST: The authors declare that there is no conflict of interests regarding the publication of this paper.

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