

EVALUATION OF *IN VITRO* ANTIOXIDANT AND ANTIDIABETIC POTENTIALS OF DIFFERENT FRACTIONS OF *MAYTENUS HEYNEANA* ROOT EXTRACTSUMITHIRA G^{1*}, SENTHIL KUMAR GP²¹Department of Pharmacology, Faculty of Pharmacy, Pacific Academy of Higher education and Research, Udaipur, Rajasthan, India.²Department of Pharmaceutical Chemistry, Bharathi College of Pharmacy, Mandiya, Karnataka, India. Email: georgesumithira@gmail.com

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ABSTRACT

Objective: In an attempt to explore herbal drug which may become useful in the prevention of diabetes and antioxidant potential by the ethanol extracts of *Maytenus heyneana* (MH) root belonging to the family Celastraceae and their different fractions were studied.

Methods: Different fractionation was done using chloroform, ethyl acetate, and methanol on ethanolic extract of MH and preliminary phytochemical analysis was done by standard methods to identify the presence of important compounds. *In vitro* antioxidants activities were carried by 2,2-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) cation radical scavenging assays. For antidiabetic potential, α -amylase and α -glucosidase enzyme inhibitory studies were carried on different fractions.

Results: Phytochemical studies show the presence of alkaloids, flavonoids, phenols, cardiac glycosides, and terpenoids in all fractionations; however, tannins and quinones were present in ethyl acetate fraction and saponins in methanolic fraction. For antioxidant activity, ethyl acetate fraction shows concentration of the sample causing 50% inhibition (IC_{50}) values in 22.31 μ g/ml and methanolic fraction shows in 12.82 μ g/ml concentrations for DPPH and ABTS radical scavenging assay, respectively. In case of antidiabetic activity, methanolic fraction offered significant result in inhibitory action of α -glucosidase and also for α -amylase assay IC_{50} (5.28 and 3.14 μ g/ml) than other fractions.

Conclusion: From the results of our studies, it can be concluded that MH shows antidiabetic and antioxidant values and methanolic fraction of MH could be possessed potential constituents in the prevention of diabetes and antioxidant than other fractions. However, further studies are required to validate.

Keywords: 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), 2,2-diphenyl-2-picrylhydrazyl, Antidiabetic, Antioxidants, *In vitro* studies, *Maytenus heyneana* root, α -amylase, α -glucosidase.

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INTRODUCTION

Diabetes, often referred as diabetes mellitus, describes a group of metabolic diseases characterized by high blood glucose, resulting from either the insulin production is inadequate, or because the body's cells do not respond appropriately to insulin, or both [1], which leads to alternations or disturbances in carbohydrate, lipid, and protein metabolism.

The global burden of DM is undoubtedly a rising from 108 million in 1980 to 422 million in 2014. The global diabetic prevalence (adults over 18 years of age) has nearly doubled since 1980, rising from 4.7% to 8.5% in 2014; this is due to the increased risk factors such as being obese and overweight. Moreover, in 2012, diabetes caused 1.5 million deaths and additional 2.2 million deaths are attributed by high blood glucose. This reflects an increase the risk of cardiovascular and other diseases. Of these 3.7 million deaths, 43% of deaths were occurring before the age of 70 years which is higher in low- and middle-income countries. The World Health Organization (WHO) states that in 2030, diabetes will be the seventh leading cause of death [2].

Diabetes is also characterized by marked postprandial hyperglycemia (PPHG) [3]. PPHG occurs when the body is not able to produce enough insulin to metabolize the glucose load that occurs 1–2 h after a meal [4]. The α -amylase and α -glucosidase are the enzymes involved in the digestion of polysaccharides and carbohydrates, which result in increased release of glucose from food sources is the main factor, affecting PPHG [5]. Acute increases in plasma glucose (hyperglycemia) concentration which promotes autooxidation of glucose to produce reactive species called as free radicals.

Long-term elevation in plasma glucose levels is associated with macro- and micro-vascular complications leads to heart diseases, stroke, blindness, kidney, and skin diseases [6]. The generation of free radicals (exogenous) beyond the scavenging abilities of endogenous antioxidant defenses results an oxidative stress [7]. Oxidative stress plays a pivotal role in the pathophysiology and progression of diabetes and its vascular complications [8]. The harmful effects of oxidative stress such as aging, anemia, arthritis, asthma, atherosclerosis, cancer, cardiovascular diseases, diabetes, hypertension, inflammation, myocardial infarction, and neurodegenerative diseases can be reduced by a constant supply of natural products [7]. The studies in recent years have shown that polyphenols in plants scavenge active oxygen species and effectively prevent oxidative cell damage [9]. The WHO survey indicated that about 70–80% of the world's population relies on alternative, complementary, and traditional medicine based mainly on plant materials for their primary health care [10] and most of which involves the use of locally available plants. The present work in aim to focus on the search of herb, which will be found more suitable among the reported herbs toward the control of blood glucose level in hyperglycemic condition based on their traditional claim of the selected herb.

Maytenus genus of flowering plants consists of about 300 species and it is distributed mainly in tropical and subtropical areas, with some found in warm temperate zones. Research shows that the extracts of different parts and isolated substances of *Maytenus* genus present a range of pharmacological effects that are antileukemia, antibacterial, anti-inflammatory, gastroprotective, analgesic, antiulcer, antidiarrheal, and antinociceptive [11]. *Maytenus heyneana* (MH) shrubs, synonym of *Gymnosporia heyneana* belongs to the family Celastraceae as shown

in Fig. 1. It is distributed southern parts (Andhra Pradesh, Karnataka, Kerala, and Tamil Nadu) of India. Conventionally, MH is used as antiarthritic [12], antidiabetic [12,13], anti-snake venom [12], immunity boosters, cancerous wound healing [12], and antidiarrhoeal [14].

The leaf of *Maytenus emarginatus*, *Maytenus putterkloides* root, and bark of *Maytenus senegalensis* have been found to possess antidiabetic activity, and also, *M. senegalensis* and *Maytenus royleanus* have reported to possess antioxidant effects. However, the root of MH (Roth.) has not been reported to antioxidant and pharmacological investigations so far. Therefore, the present study aimed to evaluate the *in vitro* antioxidant and antidiabetic activity of the root extract of MH.

METHODS

Chemicals and reagents

All the chemicals and reagents used were of analytical grade. 1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma (Sigma-Aldrich Ltd., Mumbai, India). Ascorbic acid and α -amylase were procured from LOBA CHEMIE Pvt., Ltd. (Mumbai, India). 2, 2'-azino-bis(3-ethyl benzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate, dinitrosalicylic acid, potassium phosphate buffer, and phosphate buffer saline (PBS) were received from SRL (Sisco Research Laboratories Pvt., Ltd., Mumbai, India). Acarbose and p-nitrophenyl glucopyranoside (pNPG) were obtained from TCI Chemicals Pvt., Ltd. (Chennai, India), α -glucosidase was purchased from SD Fine-Chem Ltd. (Chennai, India).

Preparation of extracts

MH roots were collected from Kolli hills of Tamil Nadu, and it was washed with clean water and air dried for 2 weeks and ground into coarse powder. Dried coarse powder (500 g) was extracted with a 90:10 (v/v) mixture of ethanol and water using Soxhlet apparatus. The resulting solvent was evaporated by rotary evaporator and dried over a water bath at a 45°C to yield the alcoholic extract of MH. Dried extract was fractionated using chloroform, ethyl acetate, and methanol. All the fractions obtained were further used for testing phytochemical analysis, *in vitro* antioxidant and antidiabetic activity.

Preliminary phytochemical analysis

Preliminary phytochemical analysis was done by standard methods [15]. Samples of different fractions of MH were tested for the presence of alkaloids, saponins, tannins, glycosides, flavonoids, phenols, steroids, terpenoids, quinones, and proteins. Results are expressed as (+) for the presence and (-) for the absence of phytochemicals.

In vitro antioxidant assay

DPPH radical scavenging assay

The antioxidant activity of the different fractions was determined in terms of radical scavenging ability or hydrogen donating, using the stable radical DPPH according to the method of Oyedemi and Afolayan [16]. The solution of 0.135 mM DPPH was prepared in methanol. Different concentration of extract (0.5 ml) was mixed with 2.5 ml of DPPH solution. The reaction mixture was vortexed

thoroughly and left in the dark room temperature for 30 min. The absorbance of the mixture was measured at 517 nm. Solution without sample was served as control and methanol served as blank. Triplicate determination was made at each dilution of sample, and the percentage inhibition of free radical scavenging capacity of the sample was calculated and expressed as concentration of the sample causing 50% inhibition (IC_{50}) values of the sample, that is, the concentration of an inhibitor where the response of DPPH is reduced by half. Ascorbic acid was used as the reference drug. The ability of plant extract to scavenge DPPH radical was calculated from the following formula:

$$\% \text{ DPPH inhibition} = \frac{\text{OD of Control} - \text{OD of test}}{\text{OD of Control}} \times 100$$

ABTS cation radical scavenging activity

The scavenging activity was conducted using the procedure Fan *et al.* [17]. A stock solution of ABTS (ABTS radical cation) was prepared by dissolving ABTS (7 mM, 25 mL in deionized water) with potassium persulfate ($K_2S_2O_8$) (140 mM, 440 μ l). The mixture was left to stand in the dark at room temperature for 15–16 h (the time required for formation of the radical) before use. For the evaluation of ABTS radical scavenging activity, the working solution was prepared by the previous solution and diluting it in ethanol to obtain the absorbency of 0.700 ± 0.02 at 734 nm. The solvent extracts and purified compounds (0.1 ml) at different concentrations were mixed with the ABTS working solution (1.9 ml) and the reaction mixture was allowed to stand at room temperature for 20 min, then the absorbance was measured using an ultraviolet (UV)-visible spectrophotometer at 734 nm. Ascorbic acid was used as the reference drug. Triplicate of sample was done and percentage inhibition for radical scavenging activity is calculated by the given equation and expressed as IC_{50} .

$$\% \text{ ABTS cation radical Scavenging} = \frac{\text{OD of Control} - \text{OD of test}}{\text{OD of Control}} \times 100$$

In vitro antidiabetic assay

α -amylase inhibitory assay

The α -amylase inhibitory activity was assessed using methods described by Fei *et al.* [18]. α -amylase was dissolved in PBS (PBS, 0.02 mol/l, pH 6.8) at a concentration of 0.1 mg/ml. Various concentrations of sample solutions (0.25 ml) were mixed with α -amylase solution (0.25 ml) and incubated at 37°C for 5 min. Then, the reaction was initiated by adding 0.5 ml 1.0% (w/v) starch substrate solution to the incubation medium. After incubation at 37°C for 3 min, the reaction was stopped by adding 0.5 ml DNS reagent (1% dinitrosalicylic acid, 0.05% Na_2SO_3 , and 1% NaOH solution) to the reaction mixture and boiling at 100°C for 5 min. After cooling to room temperature, the absorbance at 540 nm was recorded by a spectrophotometer. Acarbose was used as positive control. The result of triplicate determinations of α -amylase inhibitory activity was done and expressed as percentage inhibition by the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Abs 1} - \text{Abs 2}}{\text{Abs 1}} \times 100$$

Where, Abs1 = control and Abs2 = sample. The IC_{50} of α -amylase was calculated from its standard calibration curve.

α -glucosidase inhibitory assay

The ability of fractions to inhibit α -glucosidase enzyme was studied by the method of Ju *et al.* [19] with minor modification. The samples were reconstituted with distilled water and dimethyl sulfoxide, respectively, at various concentrations. 450 μ l of extracts were incubated with α -glucosidase (50 μ l, 0.5 units/ml) and 0.2 M potassium phosphate buffer (1500 μ l, pH 6.8) at 37°C in a water bath for 15 min. Then, 250 μ l of 3 mM pNPG was added as substrate. The reaction was incubated



Fig. 1: Photographs of (a) *Maytenus heyneana* (MH) shrubs and (b) root of MH

again for 10 min and then stopped by the addition of 750 μ l of 0.1 M Na_2CO_3 . The absorption of 4-nitrophenol, a product after reaction, was measured at 405 nm using a UV-visible spectrophotometer. Reaction mixture without the sample served as negative control and reaction mixture without the substrate served as blank. Acarbose was used as positive control. The percentage inhibition of α -glucosidase triplicate experiments was computed by equation given below and expressed as IC_{50} using standard calibration curve.

$$\alpha - \text{glucosidase inhibitory activity} = \frac{(\text{AC}^+ - \text{AC}^-) - (\text{AS} - \text{AB})}{(\text{AC}^+ - \text{AC}^-)} \times 100$$

Statistical analysis

Results of triplicate determinations were expressed as mean \pm standard deviation. Statistical analysis was done using the GraphPad Prism software version 5.0 (GraphPad Software, USA). Statistical difference between means of test sample and control sample was evaluated by one-way ANOVA followed by Turkey's multiple comparison test.

RESULTS

Preliminary phytochemical analysis

The qualitative phytochemical study on the different solvent fractions of the roots of MH showed the presence of some secondary metabolites such as cardiac glycosides, phenols, flavonoids, and terpenoids (Table 1) in all the fractions. Alkaloid was detected only in chloroform and ethyl acetate fractions and saponins were present only in methanol fraction. The presence of tannins was detected in ethyl acetate and methanol fractions except in chloroform fraction. However, steroids and proteins were absent in all the fractions.

In vitro antioxidant assay

DPPH radical scavenging assay

DPPH is composed of free radical molecules and a scavenger for other radicals, which has a deep violet color in solution and it turned to colorless or pale yellow by reduction (neutralization) of DPPH to non-radical DPPH-H in the presence of a radical scavenger (antioxidant) [20]. DPPH \cdot accepts hydrogen donation from an antioxidant and became paired, shows the scavenging ability of an antioxidant [7]. The obtained chloroform, ethyl acetate, and methanol fractions were subjected to DPPH assay in various concentrations from 5 μ g/ml to 800 μ g/ml and compared to standard, ascorbic acid. Optical density of test sample was measured at 517 nm and decreases in the absorbance pattern represent the extent of radical scavenging activity. A lower IC_{50} value corresponds with higher the antioxidant activity.

The scavenging effect increased with the increasing concentrations of test compound. The percentage inhibition values observed at 800 μ g/ml for chloroform, ethyl acetate, methanol fractions, and the standard ascorbic acid were 96.62, 97.29, 96.82, and 98.49%, respectively, and the same shown in Fig. 2.

The ethyl acetate fraction showed highest scavenging efficiency (IC_{50} =22.31 μ g/ml) when compared to other chloroform (IC_{50} =67.56 μ g/ml) and methanol (IC_{50} =30.21 μ g/ml) fractions shown in Table 2. Statistically, ethyl acetate and methanol fractions showed significant increase in DPPH radical scavenging compared with chloroform fraction ($p < 0.05$). Furthermore, It was observed that IC_{50} value also very close to standard ascorbic acid (19.14 μ g/ml).

ABTS cation radical scavenging activity

ABTS assay depends on the antioxidant compound ability to scavenge ABTS^+ , action freely soluble in both water and organic media and is produced by reacting ABTS solution with potassium persulfate. In general, ABTS^+ is rather stable [21], but in the presence of antioxidants, it reacts with hydrogen atom and is converted into non-color form of ABTS. The concentration of antioxidant in the test sample is inversely proportional to the ABTS radical formation and measurement preferably made at 734 nm.

The scavenging effect increased with the increasing concentrations of test compound. The percentage inhibition values at 200 μ g/ml for chloroform, ethyl acetate, methanol extracts, and the standard ascorbic acid were 80.22, 86.58, 89.23, and 96.4%, respectively (Fig. 3).

The ascorbic acid shows the highest scavenging efficacy with ABTS radicals (IC_{50} =6.72 μ g/ml) among all the fractions. However, methanol fraction of MH reveals greatest scavenging activity (IC_{50} =12.82 μ g/ml) and followed by chloroform (IC_{50} =43.41 μ g/ml) and ethyl acetate (IC_{50} =30.86 μ g/ml) fractions data were presented in Table 2. The radical scavenging activity of ABTS showed better activity in methanol fraction with significant difference compared to chloroform and ethyl acetate fraction ($p < 0.05$).

In vitro antidiabetic assay

α -amylase inhibitory assay

At the higher concentration of each fraction, the percentage inhibition of α -amylase recorded was 67.48, 74.08, and 76.48 % for chloroform, ethyl acetate, and methanol fractions, respectively (Fig. 4), which were considerably higher than the acarbose 64.08 %. In case of IC_{50} of

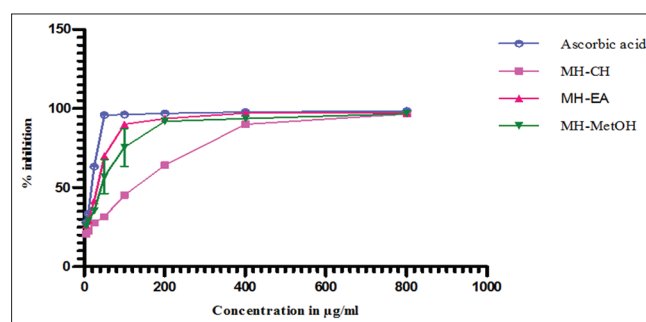


Fig. 2: Percentage inhibition of different solvent fractions Maytenus heyneana root by 2, 2-diphenyl-2-picrylhydrazyl assay

Table 1: Qualitative phytochemical constituents of different solvent fractions of MH root

S. No	Tests for	Solvent fractions		
		MH - chloroform	MH - ethyl acetate	MH - methanol
1	Alkaloids	+	+	-
2	Saponins	-	-	+
3	Tannins	-	+	+
4	Flavonoids	+	+	+
5	Phenols	+	+	+
6	Cardiac glycosides	+	+	+
7	Steroids	-	-	-
8	Terpenoids	+	+	+
9	Quinones	-	+	-
10	Protein	-	-	-

MH: *Maytenus heyneana*

chloroform, ethyl acetate, and methanol was found to be 4.11, 3.63, and 3.16 µg/ml, respectively, shown in Table 3. However, acarbose shows α-amylase inhibitory activity with IC₅₀ value of 4.44 µg/ml. Among all fractions, methanol fraction shows significantly (p<0.05) maximum inhibitory effect on α-amylase when compared with standard.

α-glucosidase inhibitory assay

Although all fractions of MH root has α-glucosidase inhibitory activity on a concentration-dependent, as observed at 5, 10, 20, 40, 80, 160 and 320 µg/ml concentrations (Fig. 5), however, the methanol fraction exhibits the highest percentage of inhibition (67.51%) among all fractions.

The IC₅₀ values of the three fractions ranged from 2.89 to 20.02 µg/ml and followed the sequence of chloroform fraction (20.02 µg/ml)>ethyl acetate fraction (12.72 µg/ml)>methanol fraction (5.28 µg/ml), indicating that the methanol fraction had the greatest inhibitory activity (Table 4). Hence, from the statistical treatment demonstrates that the methanolic fraction showed significant differences in IC₅₀ (p<0.05) compared to other fractions of chloroform and ethyl acetate.

DISCUSSION

Preliminary phytochemical analysis

Plants produce a variety of compounds can be divided into primary and secondary metabolites. Primary metabolites are essential for survival of the plant and the secondary metabolites have many beneficial uses. Secondary metabolites such as phenols, alkaloids, glycosides terpenoids, flavonoids, saponins, tannins, and carotenoids of the plant possess a wide range of various physiological and pharmacological effects on human body [22]. Therefore, qualitative phytochemical screening provides preliminary information about chemical composition of plant products [23]. In our preliminary phytochemical study showed the presence of alkaloids, saponins, tannins, flavonoids, phenols, cardiac

glycosides, terpenoids, and quinines in different solvent fractions of MH root. For instance, phenolic compounds can capture free the radicals and scavenge them and thus preventing the cells from aging process (cellular modifiers) [24]. Whereas flavonoids and saponins are reported as powerful antioxidant and anticancer activities [25]. In the same way, protective function of alkaloids also documented [25]. Similarly, cardiac glycosides used in the treatment of congestive heart failure and cardiac arrhythmia [26]. These phytochemical constituents may be responsible for the medicinal activities shown by MH and that the reason for its traditional uses as a medicine. The current study shows the presence of phenolic compounds (phenols, flavonoids, tannins, and quinones) in different solvent fractions of MH root, suggestive as a potent antioxidant. These are able to absorb the free radicals and can chelate the metal ions that which could catalyze the formation of reactive oxygen species (ROS) and thereby can promote lipid peroxidation.

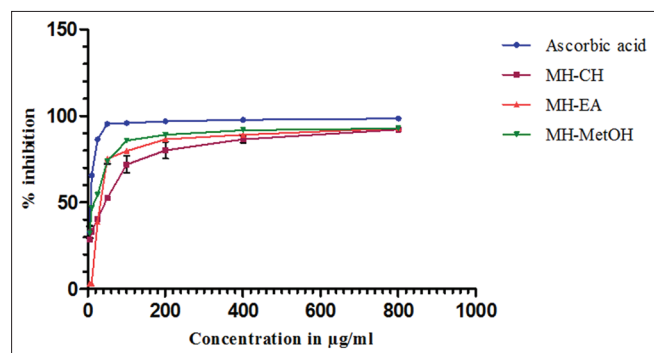


Fig. 3: Percentage inhibition of different solvent fractions *Maytenus heyneana* root by 2, 2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay

Table 2: DPPH and ABTS inhibitory effects of different solvent fractions of MH root and ascorbic acid (mean±SD, n=3)

Fractions	IC ₅₀ values of DPPH (µg/ml)	IC ₅₀ values of ABTS (µg/ml)
Ascorbic acid	19.14±0.89	6.72±0.50
Chloroform	67.56±1.04	30.83±0.45
Ethyl acetate	22.31±0.29	43.41±0.75
Methanol	30.21±1.03	12.82±0.25

Mean±SD, n=3. MH: *Maytenus heyneana*, SD: Standard deviation, DPPH: 2,2-diphenyl-2-picrylhydrazyl, ABTS: 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid, IC₅₀: Concentration of the sample causing 50% inhibition

Table 3: IC₅₀ values of the different fractions of α-amylase inhibition

Fractions	IC ₅₀ values
Acarbose	4.67±0.27
Chloroform	4.18±0.04
Ethyl acetate	3.74±0.20
Methanol	3.14±0.36

Mean±SD, n=3. SD: Standard deviation, IC₅₀: Concentration of the sample causing 50% inhibition

Table 4: α-glucosidase inhibitory effects of different solvent fractions

Fractions	IC ₅₀ values
Acarbose	02.89±0.10
Chloroform	20.02±0.16
Ethyl acetate	12.72±0.09
Methanol	05.28±0.34

Mean±SD, n=3. SD: Standard deviation, IC₅₀: Concentration of the sample causing 50% inhibition

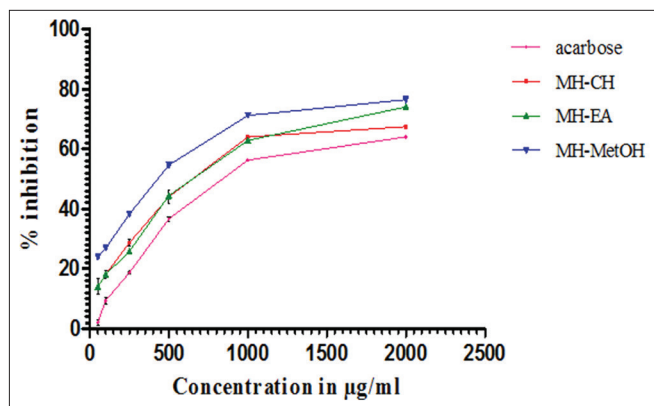


Fig. 4: α-amylase inhibition of different solvent fractions *Maytenus heyneana* root

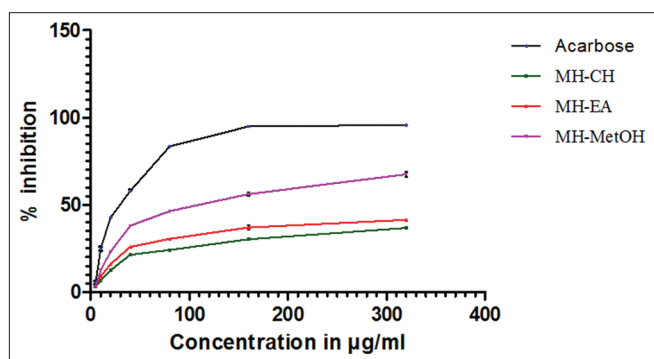


Fig. 5: Percentage of α-glucosidase inhibition of different solvent fractions *Maytenus heyneana* root

Apart from the phenolic compounds, as well the flavonoids have a great importance in fight against the ailments in the human body and also reported that plants are with alkaloids, flavonoids, phenols, glycosides, and terpenoids have antidiabetic potential [27], these reports added further evident to confirm that antidiabetic and antioxidant property of MH root.

In vitro antioxidant assay

To assess the antioxidant capacity of samples, variety of methods must be utilized in parallel, for the reason that different methods often give different results [28]. In this study, the antioxidant activity was measured using two different assays, namely DPPH and ABTS, because performing a single assay to determine the antioxidant properties would not give the correct result due to influenced by many factors, for example, the test system and composition in the different solvent fractions. The free radicals have one or more unpaired electrons which are produced during normal and pathological cell process. ROS are various form of activated oxygen which includes free radicals as well as non-free radical species (H_2O_2). Oxidative stress due to ROS may lead to number of ailments [29]. DPPH \cdot and ABTS $^{+}$ scavenging activities involve hydrogen atom transfer and electron transfer [30]. In the current study, results of scavenging of free radicals by DPPH assay exhibit that ethyl acetate fraction of MH root showed better inhibitory activity followed by methanol and chloroform fractions. In the other hand, methanol fraction showed the greatest scavenging activity by ABTS inhibitory assay. This contrary observations may be due to the different radicals have different absorption spectra and antioxidant potentials (kinetics) when reacting with phenolic compounds. This might be due to different properties. Both radicals might solve differently, their molecular size may different. ABTS radical has to be formed initially, while DPPH is really a stabilized radical by itself. Further, they might have also different affinities against other compounds that are present in the fractions.

In vitro antidiabetic assay

One of the therapeutic approaches for the treatment of diabetes is to maintain the levels of glycemic control by decrease the postprandial hyperglycemia. This can also be achieved by retarding absorption of carbohydrate. α -amylase is a key enzyme in the digestive system which involved in the hydrolyzing of starch into disaccharides and finally breaks down into glucose before absorption [31]. α -glucosidase is also another enzyme which exists in the small intestine, used for degradation of disaccharides into monosaccharide by inhibiting of these enzymes may be delay in the carbohydrate digestion which might cause a marked decrease in the rate of glucose absorption, thereby weakens the postprandial plasma glucose rise. Several inhibitors for the management of diabetes are acarbose, voglibose, and miglitol. However, these drugs are known to be associated with various gastrointestinal disturbances such as abdominal pain, flatulence, and diarrhea [32,33]. Hence, attempt made to identify and explore the amylase inhibitors from natural sources. In this study, *in vitro* effect of different solvent fractions of MH root was evaluated by α -amylase and glucosidase inhibitory assays to identify the fraction with highly capable in inhibiting such enzymes.

From the results of two assays prominent that at high concentration, methanolic fraction showed superior activity than the other two fractions, although among the different fractions, the methanol fraction showed significant increase in α -amylase activity (3.14 μ g/ml) when compared to the standard acarbose (4.67 μ g/ml) ($p < 0.05$). In case of α -glucosidase activity, also methanolic fraction shows significant differences in $IC_{50} = 5.28$ μ g/ml ($p < 0.05$) compared to chloroform (20.02 μ g/ml) and ethyl acetate (12.72 μ g/ml) fractions. This may be due to the presence of secondary metabolites such as tannins, terpenoids, and flavonoids in methanol fraction could be contributed in exerting antidiabetic activity [34]. Hence, it is evident that its possess therapeutic value in the management of diabetes.

CONCLUSION

Preliminary phytochemical studies with chloroform, ethyl acetate, and methanol fractions of MH roots showed that the presence of flavonoids, phenols, and terpenoids in all the fractions. The presence of flavonoids and phenols in fraction could be a reason for scavenge active oxygen species and effectively prevent from oxidative cell damage and also from the observed results of the current study on *in vitro* antioxidant by DPPH and ABTS assays and antidiabetic potentials by α -amylase and α -glucosidase enzyme inhibitory assays on different fractions of MH root extract, methanol fraction shown a best of activities than other fractions. Hence, it was concluded that the methanol fraction of MH root could be a good candidature for further studies and the isolation of active phytoconstituents which is responsible in the management of diabetic and as antioxidant.

AUTHORS' CONTRIBUTIONS

Sumithira G has carried out the experiment, collected the data, and drafted the article. Dr. Senthil Kumar G P, Professor cum Principal, has supervised the experiment and reviewed the article.

CONFLICTS OF INTEREST

The authors declared no conflicts of interest of this study.

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