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Antioxidative and α-amylase inhibitory potentials of medicinal plants from the Western Ghats of southern India

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ABSTRACT

Plants are important sources of chemical constituents with potential antioxidant activity and inhibitors of α-amylase and can be employed for therapeutic benefits. The leaves of medicinal plants viz., Cajanus lineata W. & A., Leucas ciliata Benth., Rauwolfia densiflora Benth. ex. Hook. and Gomphostemma heyneanum Wall., were collected from the Talacauvery subcluster of Western Ghats, India. The powdered leaves were extracted in various solvents and analyzed for the phytochemical constituents, antioxidative and α-amylase inhibitory potentials. All extracts indicated the presence of flavonoids, terpenoids, steroids and reducing sugars. Among the solvent extracts, the aqueous extract of L. ciliata leaves contained high total phenolics (105± 2.45 µg GAE/g). The solvent extracts of the leaves of this species also exhibited higher antioxidant activity. The standard ascorbic acid showed 50% inhibition at 67±1.23 µg/ml. The aqueous and methanol extracts of L. ciliata had FRAP activity of 110± 2.88 µM Fe (II)/g and 115± 3.46 µM Fe (II)/g followed by the aqueous extract of R. densiflora (82± 2.37 µM Fe (II)/g). The methanolic extract of L. ciliata showed positive results for the inhibition of α-amylase by starch-iodine color assay. Further quantification by DiNitroSalicylic Acid (DNSA) method revealed that the methanol extract of L. ciliata, showed 72.9% inhibition with IC_{50} values of 138.7± 3.47 µg/ml in comparison to the standard inhibitor Acarbose™ (IC_{50} value of 96.29± 2.89 µg/ml). The leaf extracts of L. ciliata could be used in the treatment of glucose metabolic related disorders. The study also indicates the antioxidative and anti-diabetic potentials of the plant species.

Keywords: Antioxidant assays, α-amylase inhibition, Medicinal plants, Talacauvery subcluster, Western Ghats

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INTRODUCTION

Medicinal plants have been used for centuries as remedies for human diseases due to their therapeutic benefits [1]. About 80% of the world population rely on the use of traditional medicine, which is predominantly based on plant material [2]. India is one of the twelve-mega diversity countries in the world and has 17,000 flowering plants. Among the 34 hotspots in the world, the Eastern Himalayas and the Western Ghats are the two hotspots of India [3]. The scientific studies available on a good number of medicinal plants indicate that promising phytochemicals can be developed for many human health problems [4], namely diabetes, cancer and infectious diseases. The medicinal values of plants lie in their phytochemicals such as alkaloids, tannins, flavonoids and other phenolic compounds, which produce a definite physiological action on the human body. Phytomedicines play a major role in human health care system. There is a considerable interest in elucidating the mechanism of their action to develop better medicines. Plants contain many free radical scavenging molecules such as phenolic compounds, nitrogen compounds, vitamins, terpenoids etc. Phenolics are among the most important compounds occurring in plants, comprising of least 8,000 different known structures [5]. These compounds are reported to exhibit anti-carcinogenic,

anti-inflammatory, anti-thrombotic, immune modulating and analgesic activities as antioxidants [6]. Therefore the need to develop efficient, safe and inexpensive drugs from plant sources is of great importance.

Diabetes is one of the most prevalent metabolic disorders characterized by an increase in blood sugar levels and improper primary metabolism [7]. Glucose is utilized by cells in the body for the production of energy and it takes place in the presence of hormones produced in the pancreas called insulin, in the absence of which, the glucose gets accumulated in blood and later is excreted along with the urine. Diabetes also gives rise to various secondary problems such as cataracts, micro vascular problems and neuropathy. These secondary problems commonly arise due to oxidative stress and DNA damage caused in cells by means of free radical generation [8]. It is also associated with high heart risks caused by means of improper cholesterol metabolism, which in turn leads to hyperlipidemia [9]. Hence it is necessary to identify sources of anti-diabetic as well as the one which readily overcomes the oxidative stress and hyperlipidemia problems. A therapeutic approach for treating diabetes is to decrease postprandial hyperglycemia. This can be achieved through the inhibition of carbohydrate hydrolyzing enzymes such as alpha glucosidase and alpha amylase [10, 11]. The inhibition of these enzymes is drug-design targets in the development of compounds for the treatment of diabetes, obesity and hyperlipidemia [12]. Plants have been an exemplary source of drugs and many of the currently available drugs are derived directly or indirectly from them [13]. The search for appropriate anti-hyperglycemic agents is focused on plants used in traditional medicine because natural products may be a better option than currently used drugs [14]. A number of medicinal plants and their formulations are used for treating diabetes in the traditional Indian Ayurvedic system as well as in ethnomedicinal practices. This need prompted us the current investigation.

MATERIALS AND METHODS

Collection of plant material and preparation of plant extract

Fresh and healthy leaves of *Cajanus lineata* W & A. (Fabaceae), *Leucas ciliata* Benth. (Lamiaceae), *Rauwolfia densiflora* Benth. ex. Hook. f (Apocynaceae) and *Gomphostemma heyneanum* Wall. (Lamiaceae) were collected from the natural habitats of Talacauvery sub cluster (12º 24' N to 75º 28'E; altitude: 1,276 msl) of Western Ghats, southern India and washed thoroughly in distilled water and subsequently dried in a hot air oven at 40º C for 48 hours. Thirty grams of shade dried powdered leaf material of the above mentioned plant sap were extracted successively with hexane, chloroform, ethyl acetate, ethanol, methanol and aqueous until the plant material became colorless. It was then filtered with sterile Whatman filter paper no.1 into a clean conical flask and the filtrate was transferred into the sample holder of the rotary flash evaporator (Superfit model PBU-6D, India). The residue so obtained were weighed and designated as extracts, preserved at 4º C in glass vials until further use. The yield of solvent extracts was expressed as milligram of extract per gram of fresh weight and calculated as follows: Yield = weight of dried sample (mg)/ fresh sample (g) X 100% [15].

Phytochemical investigation

Phytochemical tests were carried out on the hexane, chloroform, ethyl acetate, ethanol, methanol and aqueous extracts of above mentioned plant species using standard procedures described by Harborne [16].

Antioxidant assays

Estimation of total phenolic content

Total phenolic content in plant extracts were estimated by Folin-Ciocalteu (FC) method employing Gallic acid as a standard (1 mg/ml) as per the procedure of Volluri *et al*. [17] with some modifications. Different concentrations of standard (5-25 µg/ml) as well as the extracts (20-100 µg/ml) were taken in test tubes and one ml of FC reagent (1:1 dilution) was added. 3-5 min later sodium carbonate (20%, w/v) was added and the mixture was allowed to stand for 45 min under dark condition. After the specified incubation period, the absorbance of standard and samples were read at 765 nm in a spectrophotometer (Hitachi U-3900 UV/Visible spectrophotometer). Total phenolic content was quantified by the calibration curve obtained from measuring the absorbance of known concentrations of gallic acid standard. The total phenolic content of extracts was expressed in terms of gallic acid equivalence (mg GAE /g extract).

DPPH radical scavenging assay

Different aliquots of standard (1mg/ml) and aqueous extracts of plant sources 20-100 µg were taken and the total volume was made up to 250 µl with water/ methanol respectively. To this one ml of 1, 1- diphenyl- 2- picryl hydrazyl (4mg/100ml) was added and the tubes were kept in dark for incubation at room temperature for 20 min.

The absorbance was checked against the blank at 517 nm (Spectramax 340, Molecular Devises, USA). Percentage of free radical scavenging activity was calculated based on the extent of reduction in the color [18]. Ascorbic acid was used as the positive control. Per cent scavenging effect was determined by the following equation:

% radical scavenging = [(Absorbance of control – Absorbance of test sample)/Absorbance of control] x100

***Ferrous reducing antioxidant power assay* (*Total antioxidant activity assay*)**
The method employed was a modified method of Benzie and Strain [19]. The stock solutions consisted of acetate buffer (300 mM, pH 3.6), 2, 4, 6-tripyridyl-s-tri-azine (10 mM) solution in HCl (40 mM), and ferric chloride, hexahydrate (20 mM) solution. The fresh working solution was prepared by mixing 25 ml of the acetate buffer, 2.5 ml of TPTZ and 2.5 ml of ferric chloride hexahydrate. The temperature of the solution was raised to 37^0 C. Plant extracts (150 µL, 1mg/ml) were allowed to react with 2850 µl of the FRAP solution for 30 min in the dark condition. Readings of colored product (ferrous tripyridyltriazine complex) were taken at 593 nm (Hitachi U-3900 UV/Visible spectrophotometer). The experiment was repeated three times. Results were expressed in µM Fe (II)/g dry mass and compared with that of Butylated hydroxy toluene (BHT).

Total reducing power
The determination of reducing power was performed as described by Yen and Duh [20]. Various concentrations of extracts (20 - 100 µg/ml) were mixed with phosphate buffer (500 µl, 20 mM, pH 6.6) and potassium ferricyanide (1%, 500 µl), and incubated at 50 ^{0}C for 20 min; 500 µl of trichloro acetic acid (10%) were added, and the mixture was centrifuged at 2500 rpm for 10 min. The supernatant was mixed with distilled water (1.5 ml) and 0.1% ferric chloride (300 µl) and the absorbance was read at 700 nm. The experiment was repeated thrice. An increase in the absorbance of the reaction mixture indicated an increase in the reducing power.

α-amylase inhibition assay
Starch-Iodine color assay
Screening of plant material for α-amylase inhibitors was carried out in a 96-well microtitre plate (Tarsons®) based on the starch-iodine test [21]. The total assay mixture composed of 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride), enzyme solution and different concentrations of plant extracts. The mixture was incubated at 37°C for 10 min. Soluble starch (1%, w/v) was added to each reaction well and incubated at 37°C for 15 min. HCl (1 M, 20 µl) was added to stop the enzymatic reaction, followed by the addition of 100 µl of iodine reagent. The color change was noted. The control reaction representing 100% enzyme activity did not contain any plant extract. The known α-amylase inhibitor, Acarbose™, was used as the positive control. A dark-blue color indicates the presence of starch; a yellow color indicates the absence of starch while a brownish color indicates partially degraded starch in the reaction mixture.

3, 5-dinitrosalicylic acid assay
The inhibition assay was performed using the chromogenic DNSA method [22]. 500 µl of extract and 500 µl of sodium phosphate buffer (0.02 M, pH 6.9 with 0.006 M sodium chloride) containing α-amylase solution were incubated for 10 min at 25ºC. After pre-incubation, 500 µl of starch solution (1%) in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added to each tube at 5 sec intervals. The reaction mixtures were then incubated at 25ºC for 10 min. The reaction was stopped with 1.0 ml of dinitrosalicylic acid color reagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted after the addition of 10 ml distilled water and the absorbance was measured at 540 nm. The known α-amylase inhibitor Acarbose™ was used as a positive control. The IC_{50} values were determined from plots of percent inhibition versus inhibitor concentration and calculated by the logarithmic regression analysis from the mean inhibitory values

$$\% \text{ Inhibition} = [(A_{540\ \text{Control}} - B_{540\ \text{Extract}})]/ A_{540\ \text{Control}} \times 100$$

Data analysis
All analyses of extracts were carried out in triplicates. The reported value for each test was calculated as the mean of three measurements and represented as mean ± Standard Error of the Mean. The IC_{50} values were calculated from linear regression analysis. The results were processed by SPSS software (version 16.0).

RESULTS

Yield of extraction

The yield of extractions was expressed as weight (mg) of crude extract per gram of dried plant material as per cent yield (Table 1). Per cent yield ranged from 0.27 for the chloroform extract of *C. lineata* to 15.36 for the ethyl acetate extract of *L. ciliata*. Notably, high yields were found in *L. ciliata* solvent extracts followed by *R. densiflora, C. lineata* and lower yields were observed for *G. heyneanum.*

Table 1: Extraction yield of medicinal plants

Plant species	*Yield of extraction (%)				
	Hexane	Chloroform	Ethyl acetate	Ethanol	Methanol
C. lineata	0.68	0.27	2.47	9.51	9.36
L. ciliata	2.38	2.60	15.36	2.04	9.06
R. densiflora	4.36	2.88	1.26	12.16	2.32
G. heyneanum	0.91	1.33	0.93	2.83	1.88

**30 g of powdered leaf samples was used for extraction*

Phytochemical investigation

Qualitative analysis carried out on each plant showed the presence of various phytochemical constituents. All extracts indicated the presence of flavonoids, terpenoids, steroids and reducing sugars. Tannins were present in *C. lineata* and *L. ciliata*. Cardiac glycosides and alkaloids were present in *C. lineata, R. densiflora* and *L. ciliata*. Saponins were present in *R. densiflora, L. ciliata* and *G. heyneanum.* None of the plants had anthraquinones and phlobatannins (Table 2).

Table 2: Phytochemical investigation for the solvent extracts of plant species

Phytochemical Tests	*C. lineata*						*L. ciliata*						*G.heyneanum*						*R. densiflora*					
	H	C	EA	E	M	A	H	C	EA	E	M	A	H	C	EA	E	M	A	H	C	EA	E	M	A
Tannins	-	-	-	-	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Saponins	-	-	-	-	-	+	-	-	-	-	+	+	+	-	-	-	+	+	-	-	-	+	-	+
Flavonoids	+	+	-	-	-	-	-	-	-	+	+	-	+	-	+	-	-	-	-	-	+	-	+	+
Terpenoids	+	-	+	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	+	-	-	-
Steroids	-	+	+	-	-	-	+	+	-	-	-	-	-	+	+	-	-	+	+	+	-	-	-	+
Cardiac glycosides	+	+	+	+	+	+	-	+	+	+	+	+	-	-	-	-	-	-	+	-	+	-	-	-
Reducing sugars	+	-	-	-	-	-	+	+	+	+	+	+	-	-	+	-	+	-	-	+	+	+	+	+
Alkaloids	+	+	+	+	+	-	-	-	+	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+
Anthraquinone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phlobatannins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

"+" = Positive for the test. "-" = Negative for the test

H- Hexane, C- Chloroform, EA- Ethyl acetate, E- Ethanol, M- Methanol, A- aqueous

Antioxidant assays

stimation of total phenolic content

Among the solvent extracts of plants, aqueous (105± 2.45 µg GAE/g) and methanolic (92± 3.71 µg GAE/g) extracts of *L. ciliata* leaves contained high phenolic contents followed by the methanolic extract of *C. lineata* leaves. Low total phenolic content was found in *G. heyneanum* leaf solvent extracts and ranged from 3± 0.99 µg GAE/g to 29± 2.84 µg GAE/g. Among the solvent extracts of all plant species, the hexane extracts contained lower amount of total phenolics compared to other solvent extracts. The total phenolic content of all solvent extracts of plant species was expressed as GAE (Table 3).

DPPH radical scavenging assay

Our study clearly demonstrated that the aqueous extract of all plant species exhibited good antioxidant properties when assessed by DPPH radical scavenging assay. The percentage of DPPH decolorization is attributed to the hydrogen donating ability of test compounds. DPPH radical scavenging activity of all plant species is shown in Table 3. Aqueous (81 ± 2.78 % scavenging, IC_{50}= 32.02 ± 1.88), methanol (79 ± 1.8 % scavenging, IC_{50}= 34.41± 2.6) and ethanol (79 ± 2.8 % scavenging, IC_{50}= 35.52 ± 2.09) extracts of *L. ciliata* leaves exhibited higher antioxidant activity followed by the aqueous extracts of *C. lineata* (IC_{50}= 55.42 ±1.65) and *R. densiflora* (IC_{50}= 54.53±2.3). With the exception of chloroform extract of *L. ciliata*, the hexane and chloroform extracts of all plant

species did not indicate DPPH radical scavenging activity so also the ethyl acetate extract of *G. heyneanum*. The standard ascorbic acid showed 50% inhibition at 67±1.23 µg/ml.

Table 3: Total phenolic content, DPPH radical scavenging activity, FRAP activity and total reducing power of solvent extracts of medicinal plants

Plant species	TPC (mg GAE/g)*	DPPH (%scavenging)*	FRAP (µM Fe(II)/g)*	Reducing power (700 nm)*
C. lineata				
Hexane	12± 2.09	-	-	0.36± 0.11
Chloroform	6± 1.98	-	8± 2.09	0.98± 0.23
Ethyl acetate	16± 3.11	56 ± 2.34	26± 2.55	1.32± 0.58
Ethanol	12.5± 2.01	74 ± 3.74	27± 1.39	2.21± 0.98
Methanol	90± 2.04	68 ± 2.8	63± 3.55	4.62± 1.98
Aqueous	24± 2.89	72 ± 2.08	60± 2.73	5.00± 1.87
L. ciliata				
Hexane	4± 1.76	-	8± 1.34	0.22± 0.08
Chloroform	9.9± 2.09	63 ± 2.9	12± 1.94	1.09± 0.18
Ethyl acetate	51± 3.88	68 ± 3.02	56± 2.09	4.67± 1.09
Ethanol	80± 2.47	79 ± 2.8	58± 2.10	5.05± 1.28
Methanol	92± 3.71	79 ± 1.8	115± 3.46	5.03± 1.83
Aqueous	105± 2.45	81 ± 2.78	110± 2.88	5.18± 1.22
R. densiflora				
Hexane	5± 1.08	-	5± 1.38	0.36± 0.1
Chloroform	4.5± 2.73	-	8± 2.99	0.47± 0.11
Ethyl acetate	14± 2.37	49 ± 1.56	80± 1.89	4.69± 1.9
Ethanol	11± 3.47	72 ± 2.45	77± 2.23	4.25± 1.6
Methanol	19± 3.1	76 ± 2.89	75± 3.18	4.77± 1.78
Aqueous	39± 2.6	79 ± 1.81	82± 2.37	4.81± 2.07
G. heyneanum				
Hexane	3± 0.99	-	5± 1.99	0.28± 0.09
Chloroform	4± 1.37	-	7± 2.46	0.26± 0.07
Ethyl acetate	5± 1.08	-	40± 3.29	0.58± 0.12
Ethanol	29± 2.84	65 ± 3.09	60± 1.47	1.37± 0.87
Methanol	25± 2.36	58 ± 1.88	77± 1.04	4.51± 1.98
Aqueous	21± 1.39	63 ± 3.88	58± 2.47	4.92± 1.5

** All values are expressed as mean ±standard error means (SEM) (n=3).*

***Ferrous reducing antioxidant power assay* (*Total antioxidant activity assay*)**

The FRAP values for the extracts were lower than that of BHT (183±1.72 µM Fe (II)/g). Among the tested plant extracts, aqueous and methanol extracts of *L. ciliata* had a total anti-oxidant activity of 110± 2.88 µM Fe (II)/g and 115± 3.46 µM Fe (II)/g followed by the aqueous extract of *R. densiflora* (82± 2.37 µM Fe (II)/g). The hexane extract of *C. lineata* did not indicate FRAP activity (Table 3). Total antioxidant activity of *L. ciliata* solvent extracts ranged from 8 ± 1.34 µM Fe (II)/g to 115 ± 3.46 µM Fe (II)/g. The FRAP activity of *G. heyneanum* ranged between 5 ± 1.99 µM Fe (II)/g – 77 ± 1.04 µM Fe (II)/g.

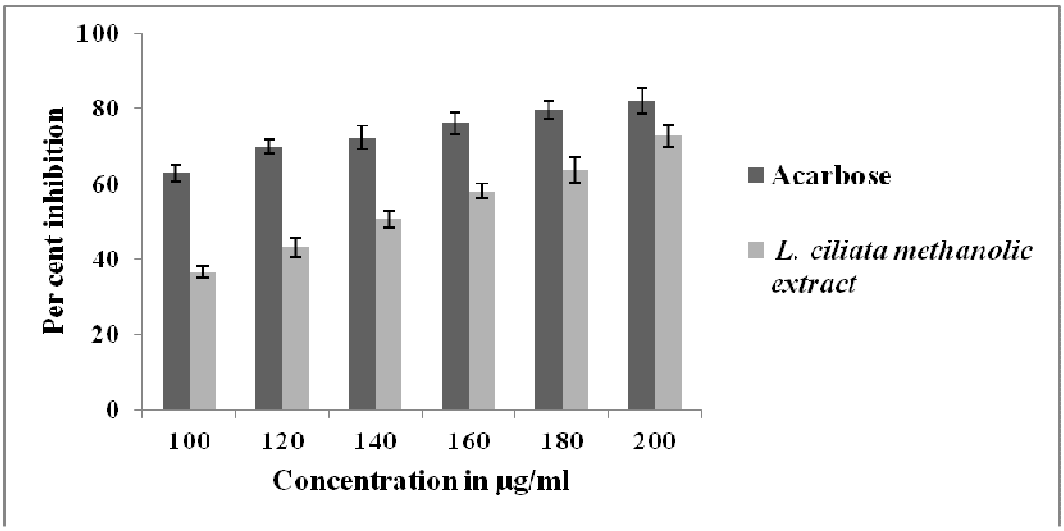
Total reducing power

The reducing power of different solvent extracts of medicinal plants using the potassium ferricyanide method is shown in Table 3. The results indicate that the reducing ability of the extracts increased with the concentration. Aqueous extracts of all plant species showed high reducing power. Among all the extracts tested for their reducing abilities, the aqueous extracts of *L. ciliata* showed high reducing power (5.18 ± 1.22 O.D.) as shown by the increasing optical density at 700 nm.

α-amylase inhibition assay

Among the solvent extracts of medicinal plants considered for the study, only the methanolic extract of *L. ciliata* showed positive results in the initial screening of inhibition for α-amylase activity by the Starch-Iodine color assay. The extract was further quantified by chromogenic DNSA method and the methanolic extract of *L. ciliata*, showed 72.9% inhibition (200 µg/ml) with IC_{50} value of 138.7± 3.47 µg/ml in comparison to the standard inhibitor Acarbose™ (82.1% inhibition with IC_{50} value of 96.29± 2.89 µg/ml) (Fig. 1).

Figure 1: Per cent inhibition of α–amylase activity of Acarbose™ and methanolic extract of *L. ciliate*



DISCUSSION

Plants known for medicinal activities are rich in secondary metabolites, which are potential sources of drugs and essential oils. The medicinal value of these plants lies in its bioactive phytochemical constituents that induce definite physiological action on the human body [23].

The rationale for extractions of plant sample from polar to non-polar solvents is to confirm and validate the phytochemical constituents, antioxidant and α-amylase inhibitory activity in the aqueous extractions performed in the traditional manner as well as to search for newer, more potent compounds in the solvent extracts. Our study revealed that sterols and saponins were absent in the ethanol extract of *L. ciliata* leaves. Qureshi *et al*. [24] reported that the phytochemical analysis of ethanolic extract of *Leucas ciliata* leaves, which indicated the presence of phenolic compounds, sterols, flavonoids, glycosides and saponins. Steroids were found to be present in all plant extracts. It has been found that some of these investigated plants contained steroidal compounds. It should be noted that the steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones [25]. Terpenoids which plays an important role in wound and scar healing process [26]. *L. ciliata* leaf extracts have been traditionally used for wound and scar healing properties.

Alkaloid was detected in *L. ciliata*, *C. lineata* and *R. densiflora* plant extracts. Saponins have been found to be potentially useful in the treatment of hyperglycemia [27]. The presence of saponins in *R. densiflora* is the reason for the usage of leaves traditionally to treat diabetes [28]. The phenolic compounds are considered like being a major group to the number of secondary metabolites that contribute to the antioxidant or free radical scavenging activity of the plants [29]. These compounds are known to act as antioxidants due to their ability to donate hydrogen or electrons and also because they are stable radical intermediates. Most antioxidant activities reported from plant sources are correlated with phenolic compounds [30-33]. Phenolic compounds undergo a complex redox reaction with phosphotungstic and phosphomolybdic acid present in the Folin–Ciocalteu reagent [34].

Our study clearly indicated that the methanolic extract of *L. ciliata* had high total phenolic content (105 ± 2.45 mg GAE/g) whereas the hexane extract of *G. Heyneanum* had a low content (3 ± 0.99 mg GAE/g). The total phenolic content of methanolic extract of *R. densiflora* was 19 ± 3.1 mg GAE/g), whereas Nair *et al*. [35] reported the total phenolic content of *R. densiflora* as 32.82 mg GAE/g. The result of the DPPH scavenging activity assay in this study indicated that the plants were potently active. Aqueous (IC_{50} value 32.02 ± 1.88), methanol (IC_{50} value 34.41± 2.6) and ethanol (IC_{50} value 35.52 ± 2.09) extracts of *L. ciliata* leaves exhibited higher antioxidant activity. This suggests that the plant extracts contain compounds that are capable of donating hydrogen to a free radical in order to remove an odd electron which is responsible for radicals reactivity. Prasad *et al*. [36] reported that the phenolics reduce DPPH radical by their hydrogen donating ability. The results obtained in this investigation revealed that the DPPH radical scavenging activities of plant species might be attributed to the hydrogen donating ability.

The total antioxidant activity of plant extract may be attributed to their chemical composition and phenolic content. Our study supports the study of Jayaprakasha *et al*. [37] who demonstrated that some bioactive compounds present in plants possessed high antioxidant activity, which was due to the presence of phenolics, carotenoids and flavonoids. Our investigation showed that the aqueous extract of *L. ciliata* with high total phenolic content (105± 2.45 µg GAE/g) also showed high values for FRAP (110 ± 2.88 µM Fe (II)/g). The reducing power is generally associated with the presence of reductones, which exert antioxidant action by breaking the free radical chain through donating a hydrogen atom [38]. In this assay, Fe^{3+}/ferricyanide complex is reduced to the ferrous form by antioxidants and can be monitored by measuring the formation of pearl's prussian blue color at 700 nm [39].

Plants with antioxidant activities have been reported to possess free radical scavenging activity [40]. Free radicals are known as major contributors to several clinical disorders such as diabetes mellitus, cancer, liver diseases, renal failure and degenerative diseases as a result of deficient natural antioxidant defense mechanism [41]. Among the plant extracts, the methanolic extract of *L. ciliata* showed 72.9% inhibition for α-amylase. Similarly, other researchers also have screened medicinal plant extracts for α-amylase activity. Funke and Melzig [42] demonstrated that the aqueous extracts of *Mitragyna inermis* and *Tamarindus indica* leaves inhibited the α-amylase activity. Sudha *et al*. [43] reported that the methanolic extract of *Morus alba* leaves and the acetone extract of *Ocimum tenuiflorum* exhibited α-amylase inhibitory activity.

The biological activities of the leaf extracts in the considered plant species have implications not only in the treatment of diabetes, but also the associated secondary disorders. The study not only involves the evaluation of anti-diabetic activity of leaf extract, but also the antioxidant activity which may pave a way to overcome the secondary problems associated with diabetes. Traditional plant remedies or herbal formulations exist from ancient times and are still widely used, despite all the controversy concerning their efficacy and safety [44], to treat hypoglycemic and hyperglycemic conditions all over the world. It must be noted that in many ethno-botanical surveys of medicinal plants used by the local population have been performed in different parts of the world and there is a considerable number of plants described as anti-diabetic. In addition, a variety of compounds have been isolated (alkaloids, glycosides, terpenes, flavonoids, etc.) but, studies need to be done to ascertain 'leads' to develop into clinically useful medicines. In the current scenario, most of modern drugs have been isolated from natural sources such as medicinal plants containing a wide range of chemical compounds that serve as leads to the development of novel anti-diabetic agents.

CONCLUSION

The leaf extracts of *L. ciliata* could be used in the treatment of glucose metabolic related disorders. The study also indicates the antioxidative and anti-diabetic potentials of the plant species.

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