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Chemical Constituents and antidiabetic activity of *Cordia dichotoma* a valuable source for bioactive compounds

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Abstract
Oxidative stress plays a major role in diabetic physiopathology; hence, the interest of using natural antioxidants as therapeutic tools exists. The aim of this study was the evaluation of *in vitro* anti diabetic and inhibitory potential of organic extracts from *Cordia dichotoma* leaves against key enzymes linked to hyperglycemia. The α-amylase and α-glucosidase inhibitory activities were investigated using an *invitro* model. Moreover, phytochemical analysis of tested extracts was carried out. The methanol fraction of this herb exhibited the IC50=168.85µg/mL and IC50=93.91µg/mL, respectively. However, the methanol fraction possessed the strongest inhibitory effect towards $^{\alpha}$-Glucosidase ((IC50 295.38 µg/ml). Furthermore, the results showed high levels of phenolic content. The results showed that this plant could be a significant source of medically important natural compounds.

Keywords: *Cordia dichotoma,* antidiabetic, antioxidant, phenols, $^{\alpha}$ -amylase, $^{\alpha}$ -glucosidase

Introduction
Medicinal plants are one of the main resources of therapeutic agents. Indeed, 80% of the world's population uses plants in health care [1]. Recently, there is an interest in the search for natural substances has considerably increased, because these substances are intended for use in foods or drugs to replace synthetic compounds, which are limited because of their side effects [2]. There is an increasing interest in using medicinal plants and their phytoconstituents as natural sources because of their well-known ability to scavenge free radicals. Effectively, plants are sources of natural antioxidants compounds that possess various pharmacological properties with little or no side effects and protect human health from many diseases [3, 5]. The prevention of oxidative stress related disease by medicinal plant products is delaying the oxidation of lipids or other molecules by inhibiting the propagation of oxidative chain reactions [2]. Phenolic compounds, one of the major classes of secondary metabolites, are getting more popular in the past few years. The popularity is due to their great potential in many biological properties. They can act as antioxidant (6) and also hypoglycemic agent. There are investigations proved that phenolic-rich plants are potential in inhibiting enzymes of $^{\alpha}$-amylase and $^{\alpha}$-glucosidase and the effect is similar to insulin functions (7). Insulin, a hormone, plays the major role to send glucose to body's cells to use for energy. But, when the pancreas (beta cells) does not release enough insulin or cells do not respond to the produced insulin, the glucose will stay in the blood. This will result in high blood sugar which is also known as diabetes. There are two forms of diabetes, type 1 (insulin-dependent) and type 2 (noninsulin dependent). The most common form is type 2 diabetes which is characterized by postprandial hyperglycemia where the blood sugar level rises abnormally after meals. Postprandial hyperglycemia can be lowered down by delaying the breakdowns of carbohydrates through inhibition of $^{\alpha}$-amylase and $^{\alpha}$-glycosidase enzymes in the digestive tract (8). For long time, the search for $^{\alpha}$-amylase and $^{\alpha}$-glucosidase inhibitors from plants has been carried out extensively because natural products have minimal side effects when compared to synthetic hypoglycemic medications such as acarbose, miglitol, voglibose, etc. They are very effective in inhibiting $^{\alpha}$-amylase and $^{\alpha}$ -glucosidase enzyme, but they can cause gastrointestinal side effects like diarrhoea bloating, and flatulence (9).
Cordia dichotoma is a species of flowering plant in the borage family, Boraginaceae. It is a medium-sized broad-leaved deciduous tree. Common names Asianplum, lasura, pidar, panugeri, naruvilli, geduri, spistanburgund dulu wanan and ntege (10). *Cordia dichodoma* leaves is used traditionally in treatment of dyspepsia, fever, ringworm, ulcers, prolapsed of uterus/vagina, headache, infection of urinary passage, diseases of lungs and spleen. The leaves, fruit, bark and seeds have been reported to exhibit antidiabetic, antiulcer, antiinflammatory, immune modulator and analgesic activites. *C. dicodoma* is used in Ayurveda and unani system of medicine for treating cold, cough and fever, skin disease. The leaves are useful as an application to ulcers and in headache. The present study aimed to investigate the antioxidant ability and inhibitory potential of *C. dichodoma* leaves on $^{\alpha}$-amylase and $^{\alpha}$-glucosidase enzymes. Furthermore, the bioactive compounds phenolic and flavonoids were being identified via UV Spectrophotometer.

Materials and Method
Chemicals and reagents
All the chemicals, reagents and solvents used in the study were of reagent grade and sometimes purified according to standard methods. The reagents and buffers namely, Folin-

Ciocalteu reagent, Griess reagent, phosphate buffer, iron-EDTAsolution, Nash reagent, acetate buffer, p-nitrophenyl-$\alpha - D$-glucopyranoside solution, dimethyl sulphoxide (DMSO).The chemicals namely, gallic acid, aluminium chloride, sodium acetate, quercetin, streptomycin, 2,2 -diphenyl-1-picrylhydrazyl (DPPH), sodium nitroprusside, ascorbic acid, riboflavin, 2,2` -azino-bis (3-ethylbenzothiozoline-6-sulfonic acid) diammonium salt(ABTS), potassium persulphate, ammonium molybdate, 2,4,6-Tri-(2-pyridyl)-s-triazine (TPTZ), hydrochloric acid, ferric chloride, acetic acid, sodium acetate, ferrous sulphate, ferrozine, sodium chloride, dinitrosalicylic acid, acarbose, α-amylase and α-glucosidase enzymes were purchased from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India), Absorbance for antioxidant and antidiabetic assays were measured on microplate reader (ROBOniK readwell Touch ELISA Plate Analyzer.

Collection of plant material and preparation of extracts

The mature fresh leaves of CD were collected from wild habitats in Thanjavur district, TamilNadu,India during January 2019. The plant were identified and authenticated by Dr. S. John Britto, The Director, the Rapinat Herbarium and centre for molecular systematics, St. Joseph's college Trichy -Tamil Nadu. India. A Voucher specimen (MM001) has been deposited at the Rabinat Herbarium, St. Josephs College, Thiruchirappalli-602 002, Tamil nadu, India. The collected leaves were washed in tap water, shade dried and ground into a fine powder. The extracts were prepared from leaf powder by Soxhalation method using three solvents namely chloroform, methanol and aqueous. The extracts were filtered through Whatman No.1 filter paper and then concentrated under reduced pressure at 40 ◦C using a rotary evaporator until solid residues were obtained. The obtained extracts were dried to remove excess amount of solvents and stored at 4 ◦C for further studies.

Yield of extraction

The calculation of the extraction yield was the weight percentage of the crude extract to the raw material (50g). The percentage of extraction yield was calculated as follows.

% Extraction yield = weight of the plant extract/ weight of the initial samplex100%

Determination of total phenolics (TPC) and flavonoids content (TFC)

The total phenolic content (TPC) was investigated by Folin Ciocalteu's method and described by Singleton et al., 1999 [11] with some modifications. Briefly, 100 µL of the gallic acid solution (standard) and samples was prepared in a 10 mL test tube. Five hundred microliter of the Folin–Ciocalteu's reagent was added and mixed. One hundred microliter distilled water was used as a blank. After that, 400 µL of 7.5% Na2 CO3 solution was added in each tube and mixed well. The samples were left to react at 25°C for 30 min in the dark room. Ultraviolet–visible (UV-Vis) spectrophotometer was used to measure the absorbance at 765 nm. Five replications were performed in each treatment. The TPC value was expressed as mg gallic acid equivalents (GAE) per g sample.The total flavonoid content (TFC) was investigated by a colorimetric method and described by Zhishen et al., 1999 [12] with some modifications. One hundred microliter of the quercetin solution and samples was prepared in a 10 mL test tube. Four hundred microliter of 2.5% NaNO2 was added and mixed. The blank used 100 µL distilled water instead of the standard solution and samples. Thereafter, 500 µL of 5% AlCl3 solution and 2000 µL of distilled water were added. The samples were allowed to react at 25°C for 10 min. The absorbance was measured using UV-Vis spectrophotometer at 415 nm. The experiments were repeated five times. The TFC value was expressed as mg quercetin equivalents (QEs) in per g sample.

GC–MS analysis of leaf extracts

Gas chromatography - Mass spectrometry analysis of leaf extracts was carried outin Shimadzu (QP2020) interfaced to a mass spectrometer. SH-Rxi-5Sil-MS non-polar, capillary column of 30 m length, 0.25 mm inner diameter and 0.25 µm film thickness and coated with 100%polydimethylsiloxane was used.Initial oven temperature was maintained at 50 ◦C and increased to 280 ◦C at a rate of 6 ◦C/min with 2 min final hold time. The injector temperature was 250 ◦C. The carrier gas was helium used at a linear velocity of 39.7 cm/sec and pressure of 68.1 kPa with a flow rate of 1.2 mL/min. One µL of leaf extracts dissolved in hexane was injected into GC with a split ratio of 1:10. Mass spectrum was obtained in electron ionization mode at 70 eV and the mass spectra were measured in a scan range from 50 to 500 amu. The ion source temperature was maintained at 200 ◦C. Interpretation of compounds were done by comparing and matching the mass spectra of each compound presentin the extract with that of standard spectra in the NIST 2005 MS library andinliterature [13]. The relativepercentage of eachcompound was measured by calculating the average peak area to total area.

***In vitro* antidiabetic activity**

α-Amylase inhibitory assay

α-Amylase inhibitory activity was executed according to Lordon *et al.*, 2013 [14]. 100µL of different concentrations of CDLE and 1% starch solution in 20 mM phosphate buffer (pH 6.9 with 6 mM sodium chloride) were incubated at 25 ◦C for 10 min. Then, 100 µL of porcine pancreatic $\alpha -$amylase enzyme (0.5 mg/mL) was added to each tube and the samples were incubated at 25 ◦C for 10 min. The reaction was stopped with 200 µL of dinitrosalicylic acid reagent and then incubated at 100 ◦C for 5 min. Once sample were cooled to room temperature, 50 µL of the reaction mixture was removed from each tube and transferred to 96 well microplate. Then the reaction mixture was diluted by adding 200 µL of distilled water in each well and absorbance was measured at 540 nm. Acarbose was used as a positive control. α-Amylase inhibitory activity of CDLE was calculated as follows, %Activity = Absorbance of extract/Absorbance of control X100

α-Glucosidase inhibitory assay

α-Glucosidase inhibitory activity was measured following the method described by (Lordon et al., 2013) [14]. Fifty µL of different concentrations of leaf extracts in 100 mM sodium phosphate buffer (pH 6.9) was mixed with 50 µL of 5 mM p-nitrophenyl--dglucopyranoside solution (in phosphate buffer) and incubated at 37 ◦C for 5 min. Phosphate buffer (100 µL) containing 0.1 µL/mL -

glucosidase was then added to each well. After 30 min, the absorbance was measured at 405 nm. Acarbose was used as a positive control. α-Glucosidase inhibitory activity of CDLE was calculated as follows, %Activity = Absorbance of extract/Absorbance of control X100

Statistical Analysis

All data were expressed as the mean standard error (± SEM) of triplicate analysis. A p-value less than 0.05 were used as the criterion for statistical significance.

Results and Discussion

The yields of extraction

The percentage of methanolic, chloroform and aqueous yields of *C. dichodoma* leaves, after extraction were (5.0%, 4.0%, 3. 7%). Most of the previous work reported higher extractives of methanol and aqueous than of chloroform (Han and Shin, 2014; Miranda et al., 2012) [11]. In contrast, the present work showed *C. dichodoma* leaves methanol yield more extractive than its chloroform.

Total phenolics and flavonoids content

The total phenolic content (TPC) in *C. dichodoma* leaf methanol, aqueous and chloroform extracts were determined by using a spectrophotometry method and a calibration curve of gallic acid as standard. The results were expressed in microgram of gallic acid equivalent (GAE) per milligram of extract (g GAE/mg extract). The results of the TPC and TFC of the CDLE are shown in (Fig. 1). It is reported that the extraction of phenolic compounds is affected by the polarity of solvents especially the quantity of polyphenols and flavonoid contents. In our study, methanol extract (1.042 mg GAE/g extract) has highest amount of phenolics, followed by aqueous (0.342 mg) and chloroform (0.091 mg) extracts. Quy Diem Do *et al.*, 2013 [12] reported a similar type of results with higher value of flavonoid content in methanolic extract from the leaves of *Limnophila aromatica*. In our study, the results were determined from a regression equation of the calibration curve (Y = 0.004 4 x + 0.031, R2 = 0.999 5) and the values were expressed as gallic acid equivalents (mg GAE/g dried extract). Nagulasamy *et al.*, 2015 [14] also reported that highest amount of phenolics is found in methanol extract than petroleum ether, acetone, chloroform and ethanol extracts of *Vaccinium leschenaultia* leaf and fruit. Phenolic compounds obtained from plant materials are act as natural antioxidants with their radical scavenging activity and reducing oxidative stress ability, they have a major role in several health benefits [2]. The highest TFC was exhibited in methanol (1.039 QE/g) extracts and least amount was recorded in aqueous (0.549 QE/g) extract. Also, there was a significant correlation between TPC and TFC (0.926, $p < 0.001$) in CDLE. The TFC of CDLE was also determined from a regression equation of the calibration curve (Y = 0.028 8x + 0.005 8, R2 = 0.999 1), and values were expressed as quercetin equivalents (mg QE/g extracts) (Fig. 1). The CDLE showed higher amount of flavonoids than methanolic and aqueous extracts of aerial parts of *Meyna spinosa* which reports with 90.08 ± 0.44 QE/g mg and 58.50 ± 0.09 mg QE/g respectively [13].

Chemical composition of *C. dichotoma* leaf extracts

The GC–MS analysis of CDLE enabled the identification of a total of 57 compounds of methanol extracts respectively were identified (Table 1) of the 57 compounds. Octadecanoic acid, 2,3-dihydroxypropyl ester, Stigmasta-7,22-dien-3-ol, acetate,(3.beta.,5.alpha.,22E), Octacosanal, Tris(2,4-di-tert-butylphenyl)phosphate 3-octyl-, methyl ester, cis-,1,1,1,3,3-Pentaisopropyldisiloxane.were found to be major constituents of the leaf. Henicosanal, Penta Fluoro propionic acid, hexadecyl ester, n-Hexadecanoic acid, Oxiraneoctanoic acid, Among the constituents, Stigmasterol (a phytosterol compound) was a significant compound present in high content (38.4%). Stigmasterol is an unsaturated phytosterol occurring in the plant fats or oils of numerous plants, such as soybean, calabar bean, and rape seed and the phytosterol content of these products is associated with their health benefits and reported to have anti-inflammatory, anti-tumor, antioxidant, antidiabetic and anti-Alzheimer's disease. Squalene is another interesting multipotent triterpenoid compound present in the leaf of CD with 36.2%. It is one of the natural antioxidants synthesized during biosynthesis of sterol from various plants and animals and has been used as a drug carrier, detoxifier and chemopreventive agent in treating various tumours related to skin, colon, breast and pancreas and possess antioxidant, anti-inflammatory, antibacterial, antifungal, cardioprotective, anticancer activities [15]. Octadecanoic acid also called Stearic acid is a saturated, wax-like, fatty acid commonly used in the production of pharmaceutical tablets and capsules. It is made by extraction from animal or vegetable fats and oils.

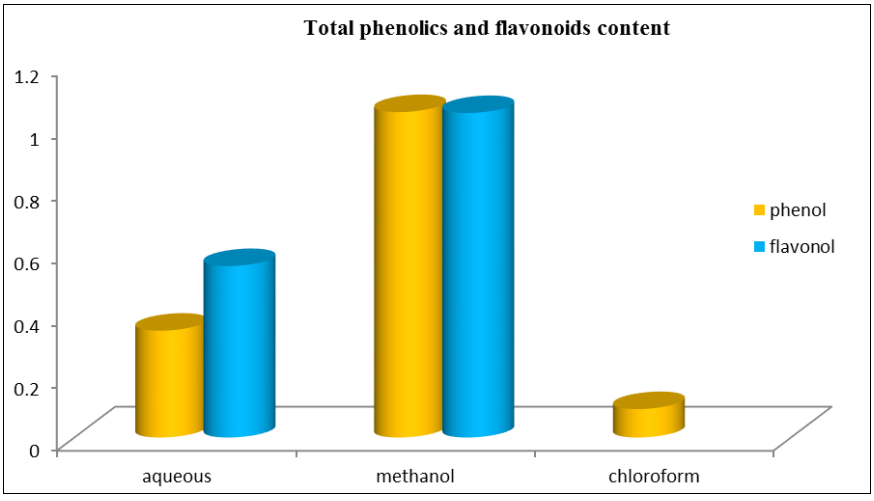

Fig 1: Total phenolics and flavonoids content of *Cordia dichotoma* leaves extracts

Table 1: Analysis the Phytochompound by GC-MS techniques - methanolic leaves extracts of *Cordia dichotoma*

R. Time compound	Area	Area%	Name of the chemical
8.110	560102	0.20	Nonane, 5-butyl-
12.339	935725	0.33	Hexadecane
13.639	949747	0.33	Hexadecane
15.502	998923	0.35	Heptadecane
16.271	1036084	0.37	Hexadecaness
17.290	898301	0.32	Octadecane
18.488	805051	0.28	Benzene, (1-Ethylundecyl)-
19.008	700035	0.25	Heptadecane
19.199	8200601	2.89	Benzene, (1-Methyldodecyl)-
19.464	2806663	0.99	Hexadecanoic acid, Methyl
19.976	1627677	0.57	Eicosane
20.351	2490915	0.88	n-Hexadecanoic acid
22.776	1345887	0.47	2-Methyltetracosane
22.965	3441099	1.21	Phytol
23.177	796212	0.28	Octadecanoic acid,
23.675	1330498	0.47	Eicosane
24.109	575344	0.20	cycloartenol
24.198	700288	0.25	Heneicosane
24.304	3597289	1.27	1-Acetoxynonadecane
25.334	2720163	0.96	Oxiraneoctanoic acid, 3-octyl-, methyl
26.155	1123548	0.40	Tetracosane
26.543	1701008	0.60	1-Acetoxynonadecanes
26.975	513963	0.18	-
27.078	615063	0.22	1,1,1,3,3-Pentaisopropyldisiloxane
27.190	2071292	0.73	2,3-Dihydroxypropyl icosanoate, 2TMS
27.267	1031944	0.36	1,2-BENZENEDICARBOXYLIC ACID
27.350	316509	0.11	Pentacosane, 13-phenyl-
27.408	2222481	0.78	Octadecane, 1-chloro-
27.480	3114885	1.10	1-(4-Undecylphenyl) ethanone
27.804	141331855	49.81	1,2-BENZENEDICARBOXYLIC ACID
27.901	284028	0.10	Tetracyclo[6.1.0.0(2,4).0(5,7)] nonan
28.109	1317301	0.46	Behenyl chloride
28.242	1139337	0.40	Octacosanal
28.343	661923	0.23	Tetracyclo[6.1.0.0(2,4).0(5,7)]nonane,
28.813	1128099	0.40	HexaethylTetracyclo[6.1.0.0~2,4~.
28.909	1780388	0.63	1H-INDOLE-3-ETHANAMINE
29.025	286908	0.10	Benzene, (1-propylheptadecyl)-
29.092	1318976	0.46	TETRADECANE
29.342	13698108	4.83	Octadecanoic acid, 2,3-dihydroxypropyl
29.593	1303361	0.46	Benzene, 2-(1-decylundecyl)-1,4-
29.775	823508	0.29	Heptacosane,
29.900	573521	0.20	TETRACYCLO[6.1.0.0(2,4).0(5,7)]NONANE
29.963	1940389	0.68	Tris(2,4-di-tert-butylphenyl) phosphate
30.150	530107	0.19	Henicosanal
30.325	898543	0.32	BENZENE, HEXADECYLPROPYL-
30.573	550688	0.19	Heneicosane
30.621	674094	0.24	Pentafluoropropionic acid, hexadecyl
31.669	3706335	1.31	Docosanal
32.127	3434820	1.21	HEXACOSANE
32.269	4682817	1.65	Stigmasta-7,22-dien-3-ol,
33.600	6703156	2.36	Heptacosanal
34.177	806539	0.28	HEXACOSANE
34.719	6253726	2.20	STIGMAST-5-EN-3-OL, (3.BETA.)-
36.296	13611283	4.80	Squalene
38.462	19279886	6.80	Stigmasterol
40.182	2766708	0.98	Octacosanal

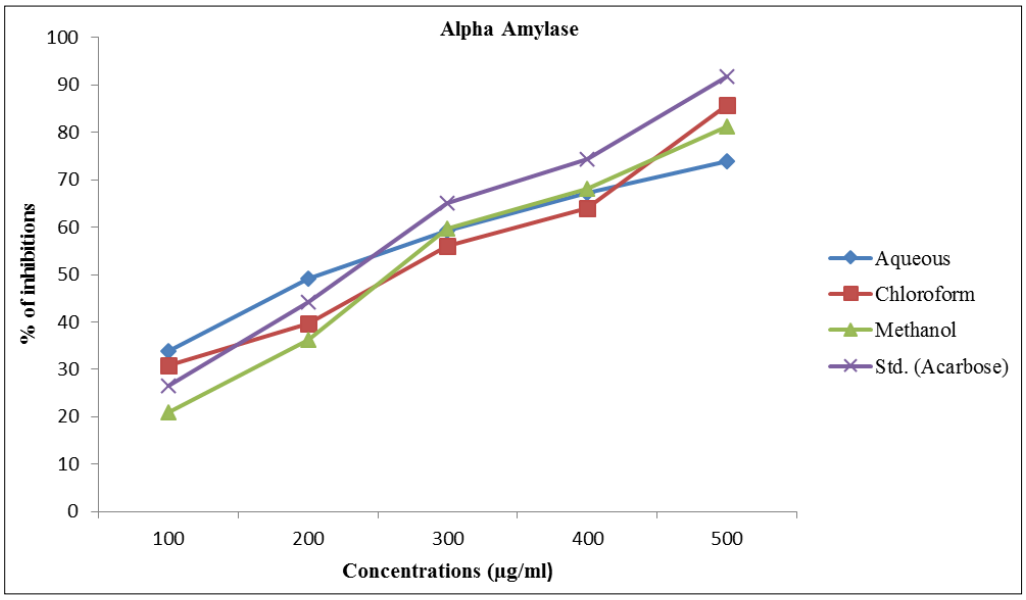

Fig 2: *In Vitro* Antidiabetic Activity of *Cordia dichotoma* leaves against Alpha-Amylase Method

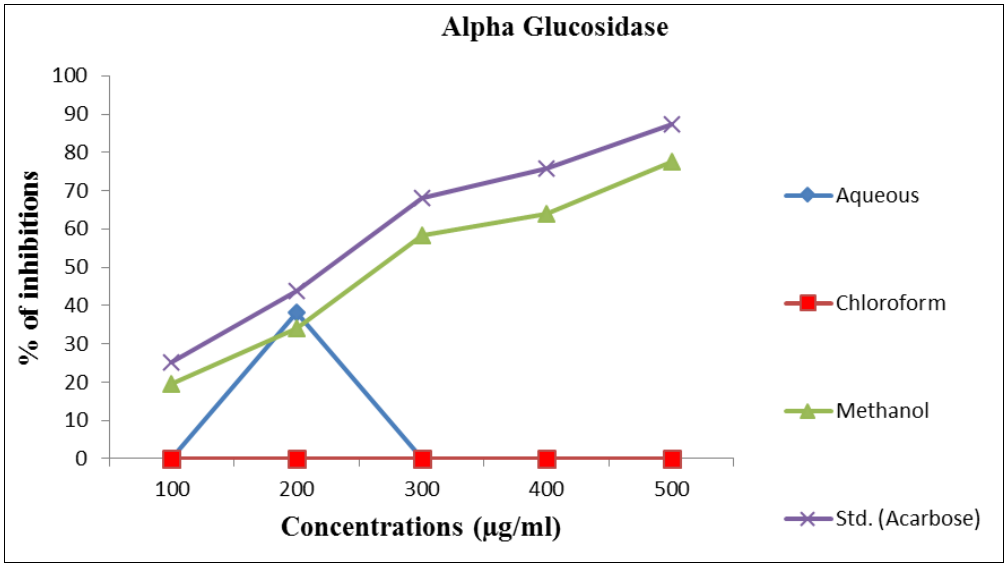

Fig 3: *In vitro* antidiabetic activity of *Cordia dichotoma* leaves against Alpha glucosidase method

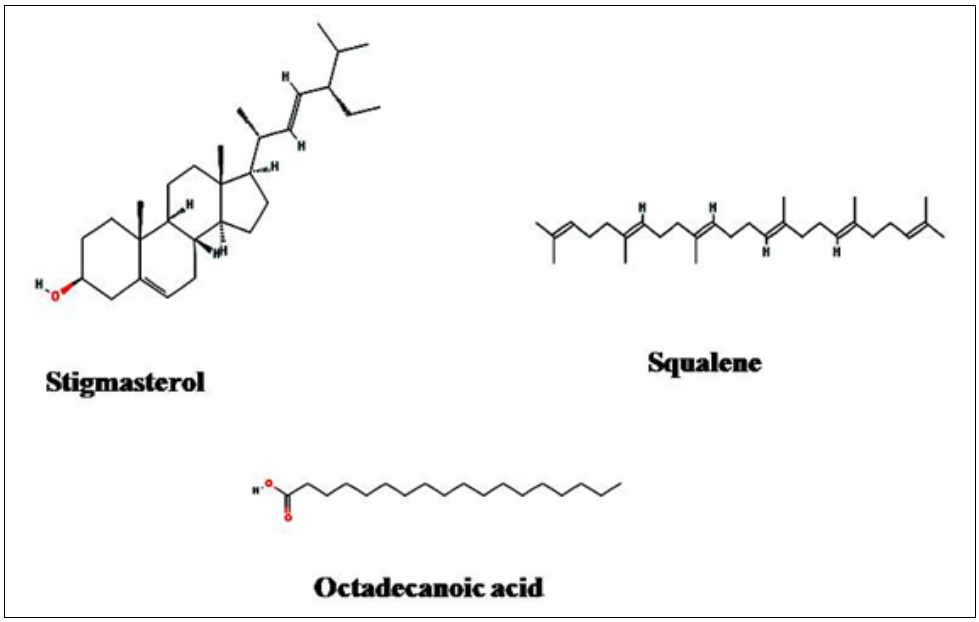

Fig 4: Chemical structure of Stigmasterol, Squalene and Octadecanoic acid

Invitro antidiabetic activity

The hydrolysis of dietary starches is the main source of glucose in the blood. The enterocytes of the small intestine can only absorb monosaccharides such as glucose and fructose from our diet. Therefore, the dietary polysaccharides need to be broken down to monosaccharides before they can be absorbed. α-Amylase catalyses the hydrolysis of α-1, 4-glycosidic linkages of starch, glycogen and various oligosaccharides and α-glucosidase further breaks down the disaccharides into simple sugars. Moreover, the α-glucosidase enzyme catalyzes the cleavage of glycosidic bond and subsequently liberates glucose from the nonreducing end of the oligosaccharide chain [12]. Acarbose is an inhibitor of α-glucosidase enzyme that commonly used to decrease glucose absorbance by reducing the production of this enzyme in the small intestine. However, this inhibitor showed some disadvantages such as increased risk of gastrointestinal problems, inconvenient dosing and costliness. Medicinal plants play an important role in the treatment of diabetes, particularly in developing countries where most people have limited resources and poor access to modern medicine

α-Amylase inhibitory assay

Methanol leaf extract of CD exhibited significant -αamylase inhibitory activity with the IC50 of 278.95 µg/mL followed by 325.50, 402.23 µg/mL for the aqueous and chloroform extracts respectively (Fig. 2). The IC50 of standard drug acarbose for -αamylase inhibition was 235. 54 µg/mL. The results of our study are also in accordance with the study by Attar et al., 2019 [17] who reported maximum -αamylase inhibition in ethanolic extract of *Lagenaria siceraria*. Methanol and aqueous extracts of CD leaves showed highest significance while, chloroform extracts showed no such difference.

α-Glucosidase inhibitory assay

In the present study, methanolic and aqueous extracts of CD showed considerable α -glucosidase inhibitory activity with the IC50 of 295.38 and 261.94 µg/mL respectively followed by 314.54 µg/mL for chloroform extracts (Fig. 3). The IC50 of standard drug acarbose for α-glucosidase inhibition was 236.05µg/mL. Likewise, aerial parts of ethyl acetate extract of *Silene salsuginea* displayed highest inhibitory effect against the α-amylase and -αglucosidase enzymes with 0.80 and 2.80 mmol ACAE/g extract respectively [17]. Sriramavaratharaajanand Murugan *et.al.*, 2018 [18] stated that, leaf essential oil of *Cinnamomum chemungianum* collected from five locations of southern Western Ghats revealed better α-glucosidase inhibition with the IC50 of 56.65 and 62.12 µg/mL for Chemungi and Athirumala accessions respectively.

Conclusion

The phytochemical analysis of CDLE led to identification of a number of secondary metabolites, in which Stigmasterol, Squalene and octadecanoic acid (Fig.4) are renowned as commercially important bioactive molecules with various pharmacological properties. To investigate the biological activities of *C. dichotoma* leaves, the antioxidant and antidiabetic activities of the methanol aqueous and chloroform extract of the plant has been analysed. As a result, we found that the extract of *C. dichotoma* have free radical scavenging activity and inhibitory activity against α-amylase and α-glucosidase and this therapeutic potentiality could be exploited in the management of post prandial hyperglycemia in the treatment of type 2 diabetes mellitus. Although the effects of *C. dichotoma* extract have been established *invitro*, these results indicate that *C. dichotoma* has potential as a crude drug and a dietary health supplement. The plant showed significant enzyme inhibitory activity, so the compound isolation, purification and characterization which are responsible for inhibiting activity, have to be done for the usage of antidiabetic agent. Further studies are also required to elucidate whether the plant have antidiabetic potential by *invivo* for corroborating the traditional claim of the plant.

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Compliance with Ethical Standards

Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Oliveira SGD, de Moura FRR, Demarco FF, Nascente PDS, Pino FABD, Lund RG. "An ethnomedicinal survey on phytotherapy with professionals and patients from basic care units in the brazilian unifed health system," Journal of Ethnopharmacology,2012:140(2):428-437.
2. Velioglu YS, Mazza G, Gao L, Oomah BD. "Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products," Journal of Agricultural and Food Chemistry,1998:46(10):4113-4117.
3. Piao MJ, Kang KA, Zhang R et al. "Antioxidant properties of 1, 2, 3, 4, 6-penta-O-galloyl-αd-glucose from Elaeocarpus sylvestris var. ellipticus," Food Chemistry,2009:(115)2:412-418.
4. Di Matteo V, Esposito E. "Biochemical and therapeutic efects of antioxidants in the treatment of Alzheimers disease, Parkinsons disease, and amyotrophic lateral sclerosis," Current Drug Targets-CNS & Neurological Disorders,2003:2(2):95-107.
5. Gerber M, Boutron-Ruault M-C, Hercberg S, Riboli EA, Scalbert M-H. Siess, "Food and cancer: state of the art about the protective efect of fruits and vegetables," Bulletin du Cancer,2002:89(3):293-312.
6. Pilarski R, Filip B, Wietrzyk J, Kuras M, Gulewicz K. Anticancer activity of the Uncaria tomentosa (Willd.) DC preparation with different oxindole alkaloid composition. Phytomedicine,2010:17:1133-1139.
7. Lee SY, Mediani A, Nur Ashiki AH, Azliana ABS, Abas F. Antioxidant and -glucosidase inhibitory activities of the leaf and stem of selected traditional medicinal plants. Int. Food Res. J,2014:21(1):165-172.

8. Hussain SA, Ahmed ZA, Mahwi TO, Aziz TA. Quercetin dampens postprandial hyperglycemia in type 2 diabetic patients challenged with carbohydrates load. Diabetes,2012:1(3):32-35.
9. Gupta RK, Kesari AN, Murthy PS, Chandra R, Tandon V, Watal G. Hypoglycemic and antidiabetic effect of ethanolic extract of leaves of Annona squaamosa L. in experimental animals. J. Ethnopharmacol. 2005:99:75-81.
10. Oudhia P, *Cordia myxa* L. [Internet] Record from PROTA4U. Schmelzer, G.H. & Gurib-Fakim, A. (Editors). PROTA (Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale), Wageningen, Netherlands, 2007.
11. Han SH, Shin SJ. Investigation of solid energy potential of wood and bark obtained from four clones of a 2-year old goat willow. Front. Energy Res,2014:2(5):1-6.
12. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Am J Enol,1999:299:152-78.
13. Adams RP. Identification of Essential Oil Components by Gas Chromatography / Quadrupole Mass Spectroscopy, 4th ed, Allured Publishing Corporation, Carol Stream, Illinois, USA, 2007.
14. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem,1999:64-555 -9
15. Thangaraj P. Pharmacological Assays of Plant-Based Natural Products, Progress in Drug Research 71, Springer, 2016, 49-55.
16. Lordan S, Smyth TJ, Soler-Vila a et al., The α-amylase and α-glucosidase inhibitory effects of Irish seaweed extracts, Food Chem,2013:141:2170–2176.
17. Attar UA, Ghane SG. *In vitro* antioxidant, antidiabetic, antiacetylcholine esterase, anticancer activities and RP-HPLC analysis of phenolics from the wild bottle ground (*Lagenaria siceraria* (Molina) Standl.), S. Afr. *J. Bot.* 2019:125:360-370.
18. Sriramavaratharajan V, Murugan R. Cumin scented leaf essential oil of *Cinnamomum chemungianum:* compositions and their *in vitro* antioxidant, -amylase, -glucosidase and lipase inhibitory activities, Nat. Prod. Res,2018:2:2081-2084.