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In vitro antidiabetic activities and GC-MS phytochemical analysis of *Ximenia americana* extracts

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ABSTRACT

Objective: To ascertain various phytochemical ingredients in *Ximenia americana* leaves extracts by GC-MS & evaluating their antidiabetic property by using *in vitro* assays.
Methods: The serial extraction was carried out with a series of solvents: chloroform, ethyl acetate, methanol, ethanol and water with increasing polarity using Soxhlet apparatus. The concentrated and dried extracts were subjected to GC-MS analysis and also the antidiabetic activity was assessed by employing standard *in vitro* techniques.
Results: GC-MS analysis confirmed the presence of different phytochemicals in each leaf extracts of *X. americana*. The major phytoconstituents were found to be Oleic acid, n-Hexadecanoic acid, Non-decanoic acid and Octadecatrienoic acid in chloroform extract; and 3-Undecene, Tridecene, Trifluoroaceteoxy tetradecene, and Trichloroacetic acid-3-tridecyl-ester in ethyl acetate extract; where in 1-Tetracosanol, Behenyl alcohol, 1-Hexacosanol, Octadecanal, 4-Piperidine propanoic acid and α –D-mannofuranoside in methanol extract; 8,11,14-Eicosatrienoic acid, 7-Tetradecanal and 1-Ocytyn-3-Ol-4-Ethy in ethanol extract; aqueous extract showed the presence of 9,12-Octadecandionoic acid. *In vitro* antidiabetic studies show that aqueous extract exhibited significant activity when compared to other solvent extracts.
Conclusion: The investigation confirms that aqueous extract exhibited highest antidiabetic activity among all extracts; Additional studies on needed for purification, characterization and structural elucidation of bioactive compounds from aqueous extract & also confirm its antidiabetic property by *in vivo* studies. This study provides scientific evidence that leaves of *Ximenia americana* have anti-diabetic efficacy. Thus, considering its relative hypoglycemic potency, they may serve as useful therapeutic agents for treating diabetes.
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1. Introduction

Diabetes mellitus is affecting around 25% of the world population of both developed and developing countries (Kayarohanam and Kavimani, 2015; Benalla et al., 2010; Rahimi, 2015). China was the country with the highest number of diabetics worldwide, with some estimated 110 million persons, followed by India (70 million) and USA (30 million) suffering from diabetes. WHO projects that diabetes will be the 7th leading cause of death in 2030. Diabetes with cardiovascular complications imposes a major threat on human health claiming death in every 10 s (Das & Rai, 2008). Diabetes cases are exponentially increasing in India as a result of societal influence and life styles (Gupta and Misra, 2007). Diabetes mellitus is considered as the group of metabolic disorders with different causes, which are characterized by imbalancing in carbohydrates, proteins and fat metabolism that lead to the effect on insulin action or secretion (American Diabetes Association, 2007). In modern medicine there is still no reasonable effective therapy or drug to cure diabetes (Ali et al., 2006). The currently accessible anti-diabetic agents include sulfonylureas, thiazolidinedione, α-glycosidase inhibitors such as miglitol and acarbose widely used to control hyperglycemia. Nevertheless, these drugs fail to cure the disease in addition, causes several diabetic complications and side effects such as abdominal pain, diarrhea and soft feces in colon (Ahmed et al., 2004; Davis and Granner, 2001).

Plant families are considered to be a source for the most potent hypoglycemic properties (Patel et al., 2012a; Patel et al., 2012b). The drugs from plant sources are usually considered to be non-toxic with lesser side effects than synthetic drugs. Traditional medicinal plants having anti-diabetic properties can provide useful sources for the discovery of safer hypoglycemic agents (Sunila et al., 2012). These plants are the major source for discovering new compounds with therapeutic value for drug development against most common and very prevalent disease, diabetes mellitus. The plants which have therapeutic application possess bioactive composites *viz*., alkaloids, glycosides, tannins, flavonoids, saponins, phenolics and vitamins. (Ghani, 2003). Different

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parts of plants vary in their composition of bioactive compounds and also medicinal properties (Harbone, 2006). Chemical compounds that are often referred as secondary metabolites are the phytochemicals formed in the plants during normal metabolic processes (Mallikharjuna et al., 2007). These secondary metabolites are an important source with a variety of structural arrangements and properties (De-Fatima et al., 2006). In ancient Indian literature medicinal properties of several herbal plants have been documented and the preparations have been found to be effective in treatment of diseases. Therefore, to come across the demand of manufacturing modern medicines and export, the need of the medicinal plants have enormously amplified (Prashanth et al., 2006).

The ethnobotanical statistics reports about 1200 odd plants that may possess antidiabetic potential worldwide (Arumugam et al., 2013; Bnouham et al., 2006; Wadkar et al., 2008; Tundis et al., 2010). In the present study *Ximenia americana* Linn. plant belonging to Olacaceae family was selected. *X. americana* is a small tree or shrub, native to

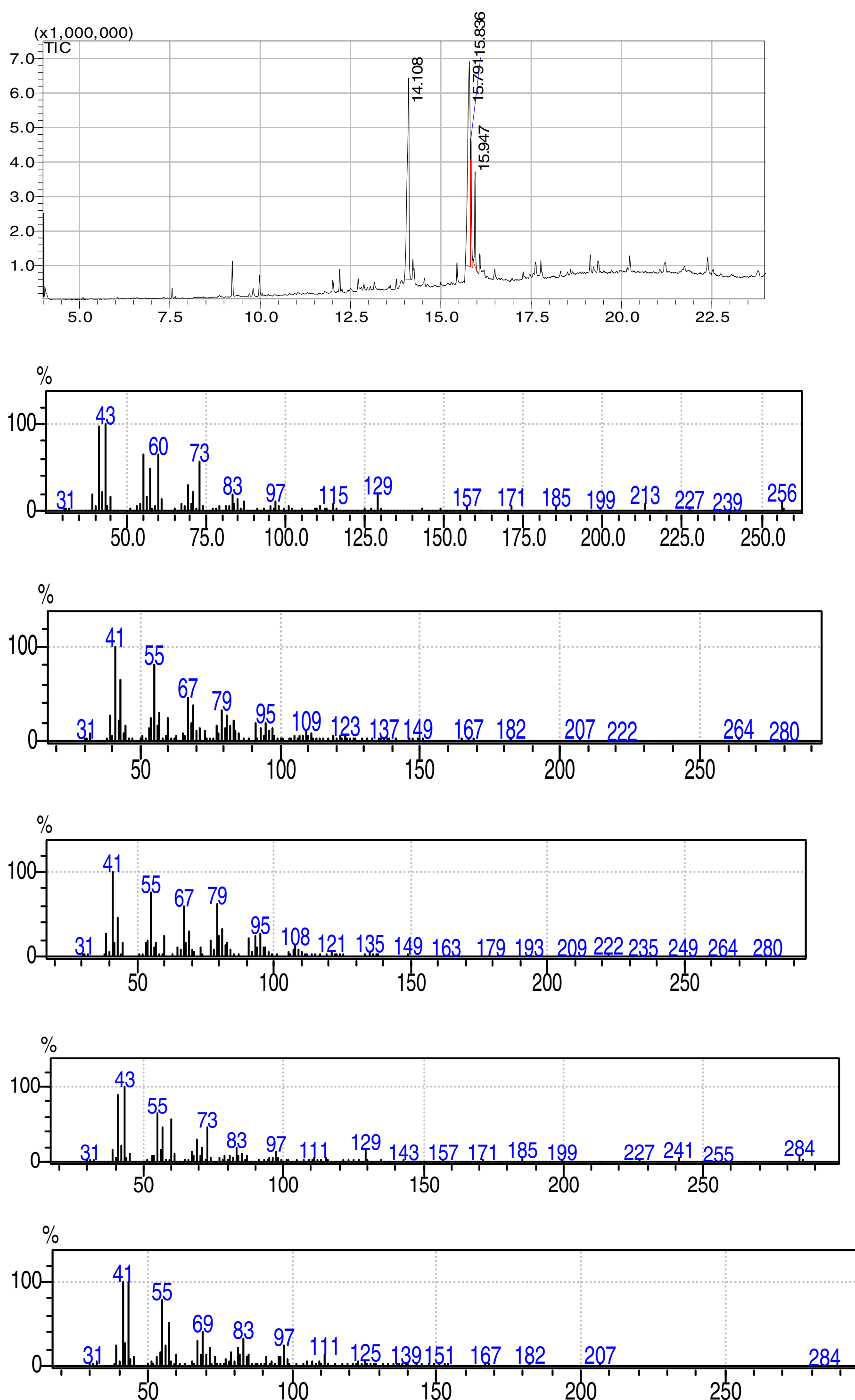

Fig. 1. GC Chromatogram of Chloroform leaf extract of *Ximenia americana*.

tropical area of Africa and seen distributed in many parts of the world. This species is used in treatment of wide variety of ailments by many rural communities in Africa and Asia. This is commonly known as wild olive or sour plum or yellow plum and extensively used as herbal remedy in treatment of malaria, leproutic ulcer, and skin infections (Voss et al., n.d.). The leaves are reported to have antibacterial activity and also used in the treatment of fever, tuberculosis, tooth decay and wounds (Ogunyleye & Ibitoye, 2003). Many investigations has validated the use of roots in the treatment of leprosy, syphilis, dysentery, and wounds. The stem bark has been reported to have anti-trypanosmal activity. The root bark and leaf of *Ximenia americana* is used as herbal medication for the cure of many ailments by Northern part of Nigeria (Maikai et al., 2008a; Maikai et al., 2008b). In our previous studies the aqueous and methanolic leaf extracts of *X. americana* showed significant antioxidant and anti-inflammatory activities (Arun et al., 2015). However, till-date a systematic study on biological activities of chemical constituents present in *X. americana* is still not agreeable (Monte et al., 2012; James et al., 2007). The extensive literature survey exposed that only few reports exist on this plant leaves, but no information are available on anti-diabetic activity in particular. Henceforth, present study was aimed to explore the phytochemical constituents of *Ximenia americana* by Gas Chromatography-Mass Spectrometry (GC-MS) analysis and also evaluating the *in vitro* anti-diabetic activity of the different solvent extracts.

2. Materials and methods

2.1. Plant collection

Ximenia americana leaves were collected from Karnatak University Campus, Dharwad, India in the month of June, 2014. The leaves were identified and authenticated by Dr. Kotresha K., Department of Botany, Karnatak Science College, Dharwad, Karnataka, India. A voucher specimen (N0-01/2016) was deposited at the Department of Botany, Karnatak Science College, Dharwad, Karnataka. Fresh disease free plant material was washed under running tap water, shade dried and

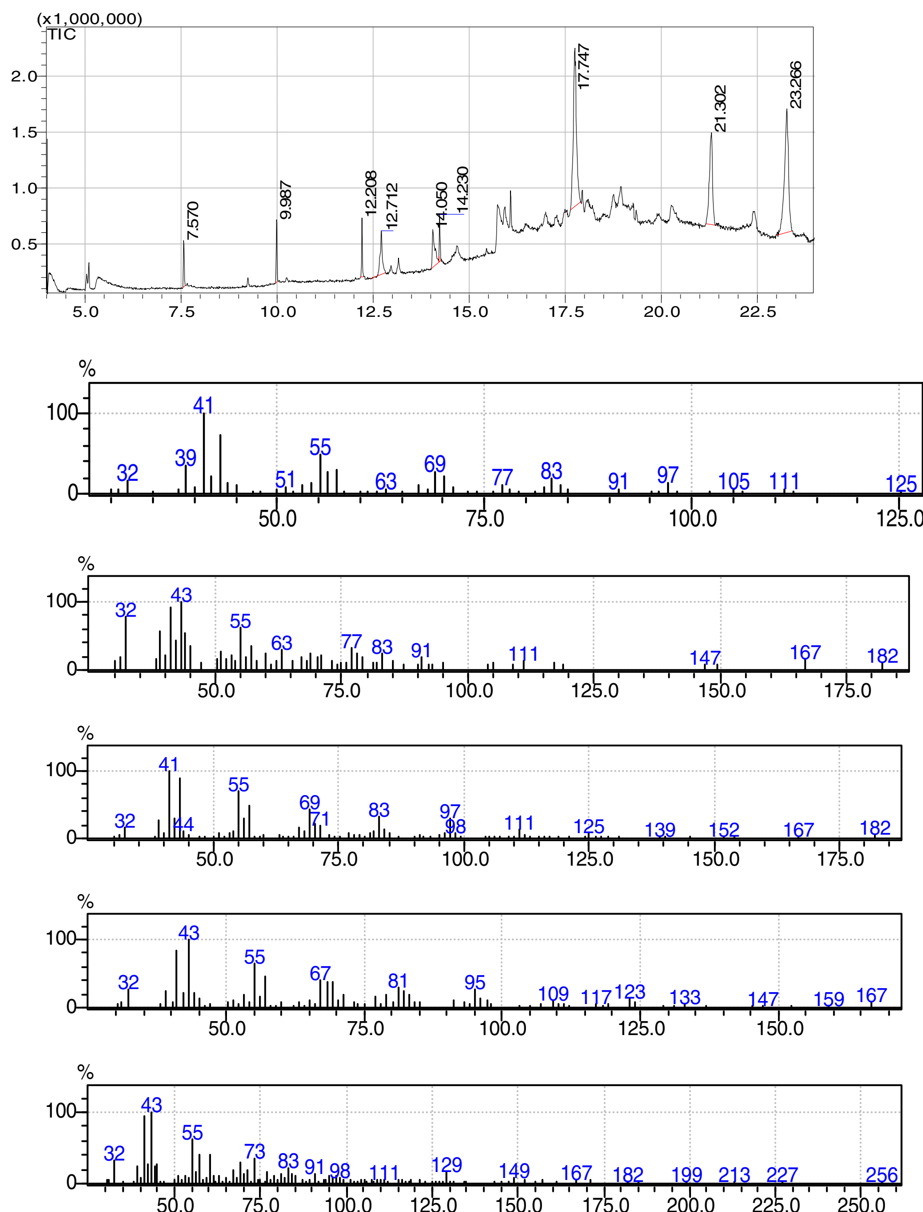

Fig. 2. GC Chromatogram of Ethyl acetate leaf extract of *Ximenia americana*.

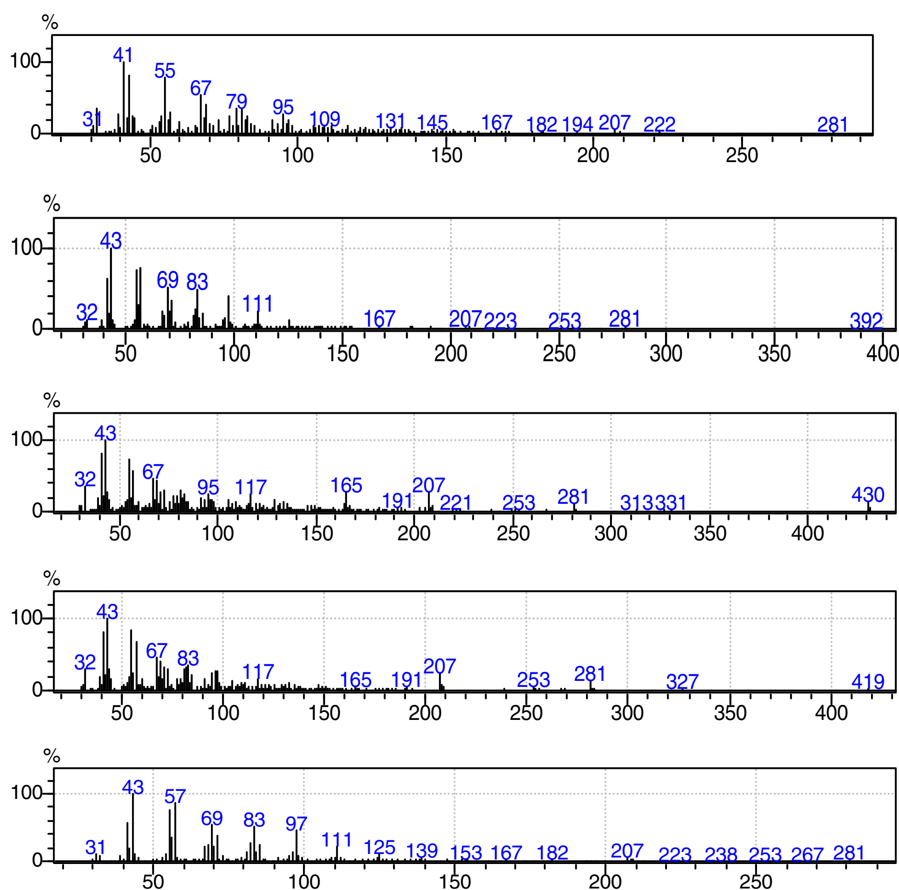

Fig. 2 (*continued*).

pulverized to fine powder using mechanical grinder. The powder was stored in airtight containers at room temperature for further use.

2.2. Crude extraction

The 100 g of dried *X. americana* leaf material was extracted with chloroform using Soxhlet apparatus for 4–6 h at 40–50 °C. The extractant solvent was evaporated using rotary evaporator and the resultant slurry of crude extract was thoroughly dried and weighed. The extract was freeze-dried at −20 °C and stored at 4 °C until use. Similarly, ethyl acetate, methanol, ethanol and water extracts were obtained. The yield was found to be 4, 5, 3.7, 4 and 8% w/w respectively with reference to the air dried plant material. All extracts were concentrated in desiccator and subjected to GC-MS analysis.

2.3. GC-MS profiling of Ximenia americana extracts

GC-MS model GCMS-QP2010S was used in the analysis that employs fused silica column and the components were separated using helium as a carrier gas at a constant flow of 1 ml/min. The 1 μl sample extract was injected into the instrument. The initial temperature was set at 100 °C, whereas the injector temperature was set at 250 °C and throughout the process temperature flow was set at the speed of increasing 10 °C/min. The actual separation was observed at 24th minute, for which final temperature was adjusted to 280 °C and run for 5 min (Gopalakrishnan & Vadivel, 2011).

2.4. Mass spectral interpretation and identification of compounds

Identification of the compounds was done based on comparison with library spectra data bases of National Institute of Standard and Technology (NIST). The spectrum of the unknown component was compared with spectrum of known components stored in the NIST library. The structure of components in the test materials were identified based on the molecular weight. The name, molecular formula, molecular weight, compound nature of test material was identified based on literature and NIST library (Kirtikar and Basu, 2001; Davies, 1990). Biological activity of all identified compounds was identified by literature survey and Dr. Duke's Phytochemical and Ethno botanical Databases (Dr. Duke's Phytochemical and Ethnobotanical Databases).

2.5. Evaluation of antidiabetic activity by using in vitro assays

2.5.1. Alpha-amylase inhibitory assay

The Alpha-amylase inhibitory assay of methanol and aqueous extracts of *X. americana* was evaluated according to a previously described method by Ranilla et al. (2008) with slight modification (Lena Galvez Ranilla et al., 2008). In brief, 0.5 ml of extract was mixed with 0.5 ml of α-amylase solution (0.5 mg/ml) with 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl). The mixture was incubated at room temperature for 10 min and 0.5 ml of starch solution (1%) in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added. The resulting mixture was incubated at room temperature for 10 min, and the reaction was terminated using 1 ml of dinitrosalicylic acid color reagent. At this time, the test tubes were placed in a water bath (100 °C for 5 min) and cooled until room temperature was attained. The mixture was then diluted with 10 ml of deionized water, and absorbance was determined at 540 nm. The absorbance of blank (buffer instead of extract and amylase solution) and control (buffer instead of extract) samples were also determined. Acarbose was used as standard drug. The inhibition of α-amylase was calculated using the following

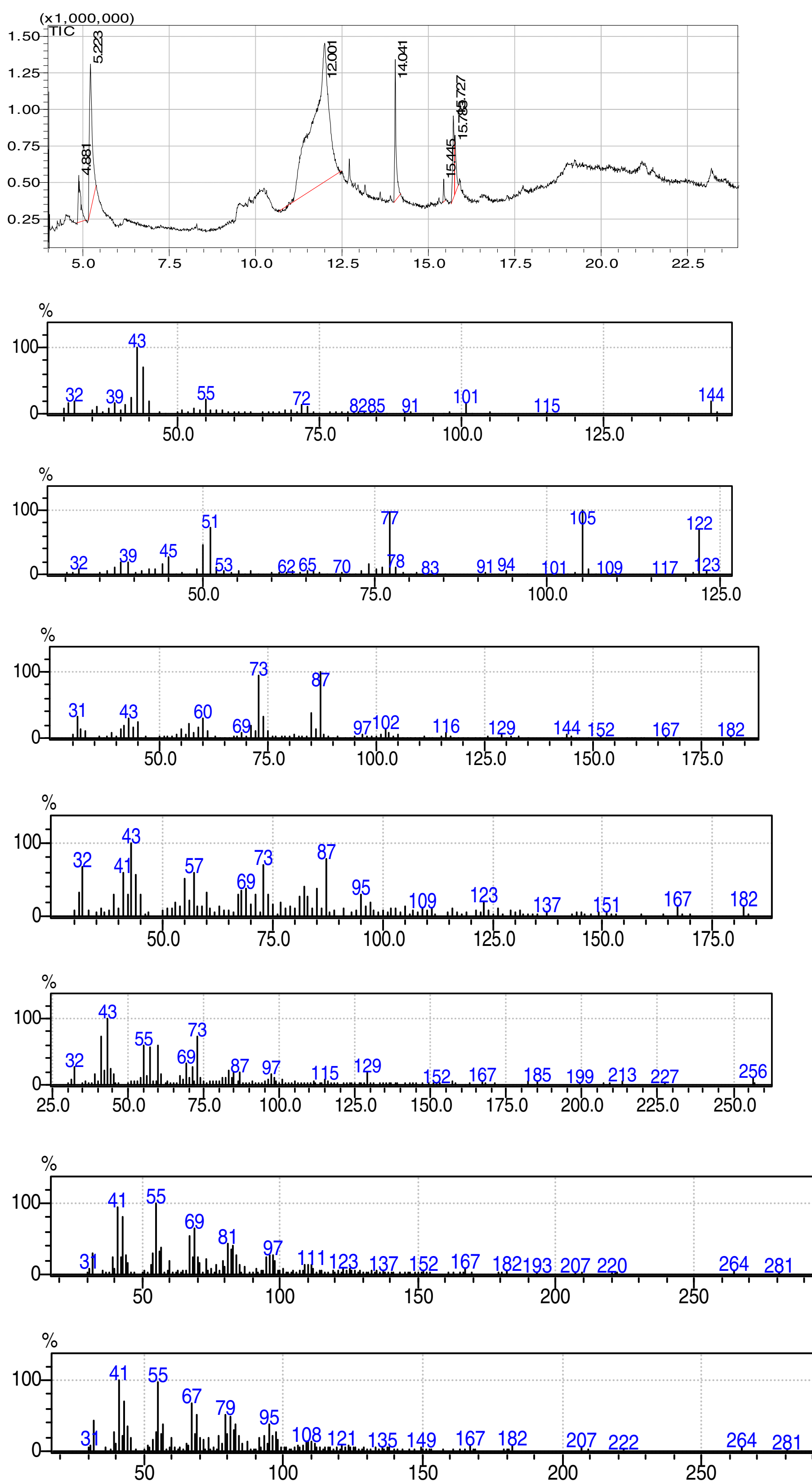

Fig. 3. GC Chromatogram of Methanol leaf extract of *Ximenia americana*.

equation:

$$\%\ \text{inhibition of}\ \alpha-\text{Amylase} = (\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})/(\text{Abs}_{\text{control}}) \times 100$$

where $\text{Abs}_{\text{control}}$ corresponds to the absorbance of the solution without extract (buffer instead of extract) and with α -amylase solution and $\text{Abs}_{\text{sample}}$ corresponds to the solution with extract and α -amylase solution.

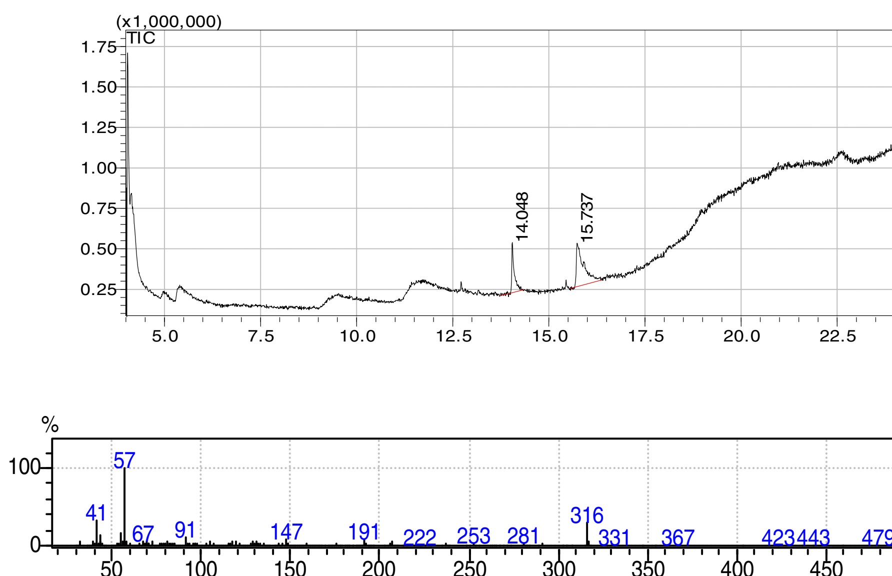

Fig. 4. GC Chromatogram of Ethanol leaf extract of *Ximenia americana*.

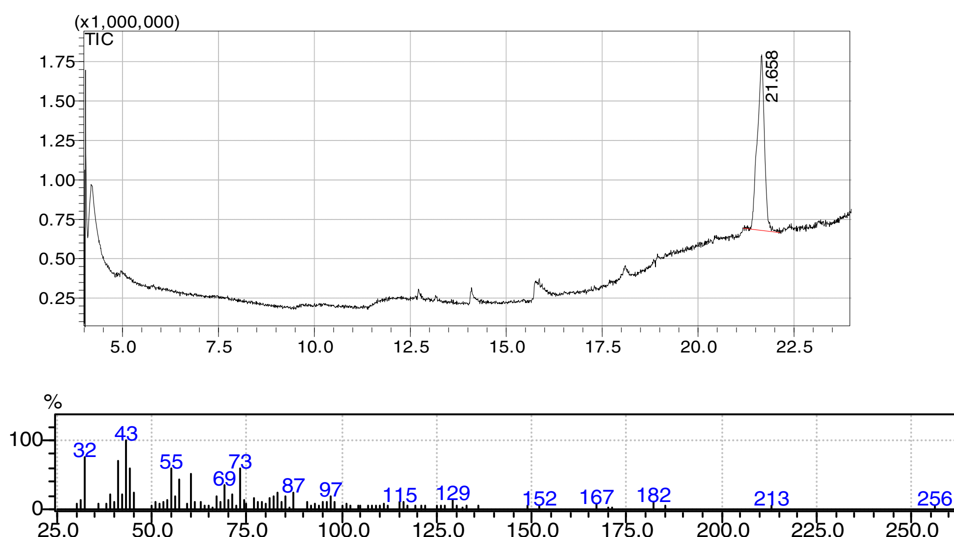

Fig. 5. GC Chromatogram of Aqueous leaf extract of *Ximenia americana*.

2.5.2. Glucose uptake in yeast cells

Glucose uptake assay by yeast cells was performed according to Cirillo et al. (1963) (Cirillo et al., 1963). The yeast, *Saccharomyces cerevisiae* suspended in distilled water was subjected to repeated centrifugation (3000 × *g*, 5 min) until clear supernatant fluids were obtained and 10% (v/v) of the suspension was prepared in distilled water. Various concentrations of plant extracts (50 to 250 μg/ml) were added to 1 ml of glucose solution (5 mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100 μl of yeast suspension followed by vortexing and further incubation at 37 °C for 60 min. After 60 min, the tubes were centrifuged (2500 × *g*, 5 min) and amount of glucose was estimated in the supernatant. Metronidazole was used as standard

Table 1
Phytochemical compounds identified in the chloroform extract of *Ximenia americana* using GCMS.

Sl. no.	Compound name	Molecular formula	Molecular weight	Retention time	Peak area	Similarity index	Compound nature	Uses
1.	Oleic acid	$C_{18}H_{34}O_2$	282	15.791	45.64	95%	Monounsaturated fatty acid	Anti-oxidant and anti-Breast cancer.
2.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	14.108	35.29	93%	Saturated fatty acid	Cosmetic, anti-oxidant, anti-bacteria and anti-fungal.
3.	Non-decanoic acid	$C_{19}H_{38}O_2$	298	15.836	11.21	91%	Fatty acid	Dietary nutrient, anti-inflammatory, Biomarker for Prostate cancer.
4.	Octadecatrienoic acid	$C_{18}H_{30}O_2$	278	15.947	7.86	90%	Fatty acid	Dietary nutrient and anti-inflammatory.

Table 2
GC–MS analysis of phytochemical compounds in the Ethyl acetate extract of *Ximenia americana*.

Sl. no.	Compound name	Molecular formula	Molecular weight	Retention time	Peak area	Similarity index	Compound nature	Uses
1.	3-Undecene	$C_{11}H_{22}$	154	7.57	3.34	92%	–	–
2.	Tridecene	$C_{13}H_{26}$	182	9.987	4.65	91%	Fatty acid	Respiratory irritations.
3.	Trifluoroaceteoxy tetradecene	$C_{16}H_{29}F_3O_2$	310	14.050	8.24	89%	–	–
4.	Trans-11Tetradecenylacetate	$C_{16}H_{30}O_2$	254	12.712	5.42	87%	–	–
5.	Trichloroacetic acid-3-tridecyl-ester	$C_{15}H_{27}C_{13}O$	344	12.208	4.03	87%	–	–

Table 3
Compound identified in the methanol extract of *Ximenia americana* using GCMS.

Sl. no.	Compound name	Molecular formula	Molecular weight	Retention time	Peak area	Similarity index	Compound nature	Uses
1.	1-Tetracosanol	$C_{24}H_{50}O$	354	5.223	13.42	93%	Fatty acid alcohol	–
2.	Behenyl alcohol	$C_{22}H_{46}O$	326	12.001	73.34	92%	Saturated fatty alcohol	Anti-viral agent (Herpes simples virus)
3.	1-Hexacosanol	$C_{26}H_{54}O$	382	14.041	5.21	91%	Saturated fatty alcohol	Wax and Polymer
4.	Octadecanal	$C_{18}H_{36}O$	268	15.445	0.69	90%	Long chain aldehyde	Pheromone
5.	4-Piperidine propanoic acid	$C_{19}H_{26}ClNO$	351	15.727	4.27	89%	–	–
6.	α –D-mannofuranoside	$C_7H_{14}O_6$	194	15.783	2.63	86%	Carbohydrate	Thickening agent, plasticizer, and resins.

drug. The percentage increase in glucose uptake by yeast cells was calculated using the following formula:

$$\text{Increase in glucose uptake }(\%) = \frac{\text{Abs}_{\text{sample}} - \text{Abs}_{\text{control}}}{\text{Abs}_{\text{sample}}} \times 100$$

where, Abs sample is the absorbance of test sample and Abs control is the absorbance of control reaction (containing all reagents except the test sample). All the experiments were carried out in triplicates.

2.6. Statistical analysis

All experiments were performed in triplicates ($n = 3$) and the data are presented as the mean ± standard error. Differences between the means of the individual groups were analyzed using the analysis of variance procedure of SPSS software Version 20 (IBM). The significance of differences was defined at the $p < 0.05$ and $p < 0.01$ level.

3. Results

3.1. GC-MS result

The results pertaining to GC-MS analysis (Figs. 1–5) revealed the identity of four major compounds present in chloroform extract, five in the ethyl acetate extract, six in the methanol extract, three in the ethanol extract, and only one in the aqueous extract of *Ximenia americana* leaves. These compounds were identified through mass spectra generated by mass spectrometry connected to GC. The various compounds present in the leaf of *X. americana* were detected by the GC-MS as shown in Tables 1–5.

3.2. Alpha-amylase inhibitory assay

The different solvent extracts of *X. americana* were subjected to α-amylase inhibitory assay along with Metronidazole as a standard. The aqueous extract showed higher activity among all other extracts tested (Fig. 6), which was comparable to standard acarbose. The α-amylase inhibitory activities of differed solvent extracts are recorded in Table 6.

3.3. Glucose uptake in yeast cells

Different concentrations of *X. americana* leaves solvent extracts are subjected to *in vitro* glucose uptake assay employing yeast as model. The percentage of glucose uptake in yeast cells by the extract was compared with standard drug metronidazole. Aqueous extract exhibited higher activity than the remaining solvent extracts tested (Fig. 7). There was concentration dependent increase in percentage of glucose uptake with increasing in concentration of *X. americana* extract (Table 7). Among all the extracts studies, aqueous extract exhibited highest percentage of glucose uptake *i.e.* 67.08 ± 0.499, which was almost near to the standard *i.e.* 68.06 ± 0.496 (Fig. 7) at 250 μg concentration. Results also indicated that *X. americana* had almost same efficiency in increasing the glucose uptake by yeast cells as compared to standard drug metronidazole.

4. Discussion

Diabetes mellitus is a non-communicable disease often genetic in nature but can be developed due to the life style. In modern medicine there is no acceptable effective therapy or medication to treat diabetes (Ali et al., 2006). Medicinal plants having anti-diabetic properties can provide a useful source for the unearthing of safer economic anti-diabetic drug. Recent extensive review by Benalla et al. (2010) listed 47 species that belong to 29 plant families as a source of alpha glucosidase inhibitors (Benalla et al., 2010), but *X. americana* was not listed. In the present research investigation different solvent extracts of *X. americana* are evaluated for their anti-diabetic activity. Two different *in vitro* assays were used to evaluate anti-diabetic activities of different solvent extracts of *X. americana viz*., alpha-amylase and glucose uptake assay.

Table 4
Compound identified in the Ethanol extract of *Ximenia americana* using GCMS.

Sl. no.	Compound name	Molecular formula	Molecular weight	Retention time	Peak area	Similarity index	Compound nature	Uses
1.	8,11,14-Eicosatrienoic acid	$C_{20}H_{34}O_2$	306	14.048	30.18	92%	Polyunsaturated fatty acid	Prostaglandins
2.	7-Tetradecanal	$C_{14}H_{26}O$	210	15.737	69.02	89%	Alcohol	Alcohol Industry
3.	1-Ocytyn-3-Ol-4-Ethy	$C_{10}H_{18}O$	154	15.783	0.80	81%	Ester	Perfume flavor and Food additives

Table 5
Compound identified in the aqueous (water) extract of *Ximenia americana* using GCMS.

Sl. no.	Compound name	Molecular formula	Molecular weight	Retention time	Peak area	Similarity index	Compound nature	Uses
1.	9,12-Octadecandionoic acid	$C_{18}H_{32}O_2$	280	21.658	100	86%	Poly-unsaturated fatty acid	Anti-oxidant, surfactant and Oil paints

Alpha-amylase is type of the intestinal enzyme which play important role in carbohydrate digestion and glucose absorption. Suppression of the activity of digestive enzymes such as α-amylase, would delay the digestion of starch and oligosaccharides, which in turn decreases the absorption of glucose and consequently reduce the blood glucose (Puls et al., 1997). Aqueous extract of *X. americana* exhibited highest percentage of inhibition *i.e.* 51.94 ± 0.265 (Fig. 6). This significant anti-diabetic activity was comparable to the standard drug inhibition. This technique is one of the anti-diabetic therapeutic approaches to reduce the post prandial glucose level in blood by inhibiting activity of alpha-amylase enzyme and it can be used as a strategy in management of blood glucose.

Regulation of glucose level in the blood of diabetic patient can prevent numerous complications associated with the disease. The maintenance of plasma glucose concentration for longer time under variation in dietary condition is one of the most important and closely regulated processes observed in the mammalian species (Ammayappan et al., 2012), especially type II diabetes characterized by deficiency of insulin causing increased level in blood glucose level and it depends on the uptake of glucose by the cells (Shori, 2015). In the present study, different solvent extracts of *X. americana* were subjected to *in vitro* anti-diabetic assay by means of yeast as model. Percentage of increase in glucose uptake in yeast cells by the action of *X. americana* extracts was compared with the standard drug metronidazole. The increased concentration of extracts correspondingly increased percentage of glucose uptake in yeast cells. This result indicated that high concentrations of extracts exhibited high glucose uptake.

Plants are the major source for discovering new compounds with medicinal value for drug development (Bnouham et al., 2006; Wadkar et al., 2008; Tundis et al., 2010 and Shori, 2015). In chromatography methods, gas chromatography (GC) is one of the most widely used techniques and has become one of the most important tools for the separation of phytocompounds. In the last few years GC-MS has become firmly established itself as a powerful technique for identification of secondary metabolites in both plant and non-plant species (Sharma and Vijayvergia, 2015; Robertson, 2005; Fernie et al., 2004; Kell et al., 2005). In the present study different solvent extracts of *X. americana viz*., chloroform, ethyl acetate, methanol, ethanol and aqueous extracts were subjected to GC-MS analysis. GC-MS spectrum confirms the presence of various bioactive compounds in all extracts of *X. americana* with different retention time. The gas chromatogram shows presence of relative concentration of various compounds present in *X. americana* getting eluted at different retention time (Hema et al., 2011a; Praveen et al., 2010; Maruthupandian and Mohan, 2011; Grover et al., 2002; Hema et al., 2011b). The peak height represents relative concentration of components present in *Ximenia americana*. The mass spectrometer helps in the identification of compounds eluted at different retention time (Figs. 1–5). The major phytoconstituents were found to be *Oleic acid*, *n-Hexadecanoic acid*, *Non-decanoic acid and Octadecatrienoic acid* in chloroform extract; and *3-Undecene*, *Tridecene*, *Trifluoroaceteoxy tetradecene*, *Trans-11Tetradecenylacetate and Trichloroacetic acid-3-tridecyl-ester* in ethyl acetate extract; where in *1-Tetracosanol*, *Behenyl alcohol*, *1-Hexacosanol*, *Octadecanal*, *4-Piperidine propanoic acid and α –D-mannofuranoside* in methanol extract; *8,11,14-Eicosatrienoic acid*, *7-Tetradecanal and 1-Ocytyn-3-Ol-4-Ethy in ethanol extract*; aqueous extract showed the presence of *9,12-Octadecandionoic acid*. The above mentioned identified compounds are known to possess several biological activities and industrial applications such as n-Hexadecanoic acid was known to have antioxidant, anti-inflammatory, hypocholesterolemic and cancer prevention activities (Kalpana et al., 2012); tetradecanoic acid was known to have antibacterial and antifungal activity (Agoramoorthy et al., 2007); *9,12-Octadecandionoic acid was* reported to have anti-inflammatory, antibacterial, hypocholesterolemic and hepatoprotective activity (Sermakkani & Thangapandian, 2012); oleic acid was proven to having antibacterial activity and industrial application as emulsifying agent (Smolinske & Susan, 1992). Overall these phytoconstituents have been shown to possess different biological activities and industrial applications such as antioxidant, anti-inflammatory, antiviral, antibacterial, antifungal, dietary nutrient,

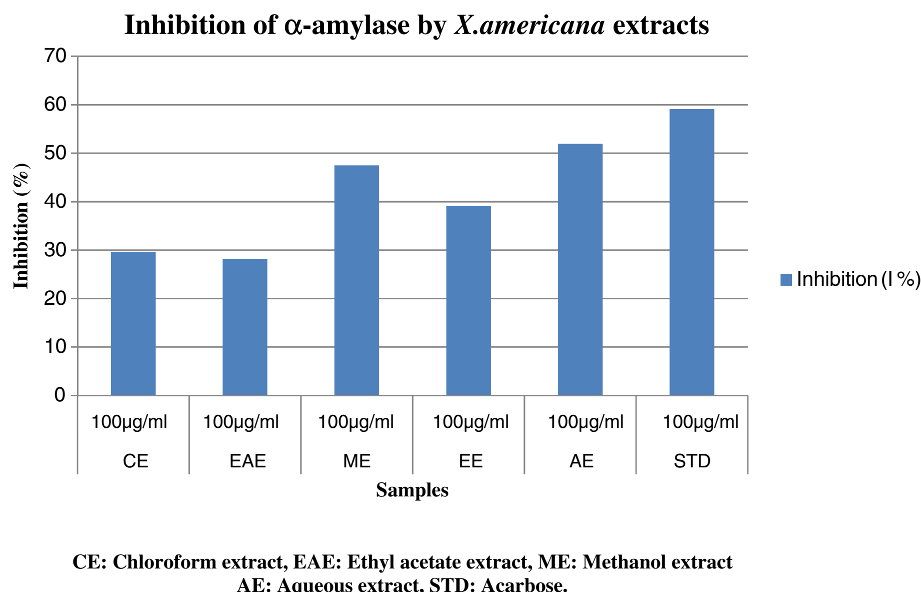

Fig. 6. Percentage inhibition of α-amylase by *Ximenia americana* leaf extracts CE: Chloroform extract, EAE: Ethyl acetate extract, ME: Methanol extract AE: Aqueous extract, STD: Acarbose.

Table 6
α-Amylase inhibitory activities and IC50 values by *Ximenia americana* extracts.

Samples	Concentration	Inhibition (I %)	IC50 (μg/ml)
Chloroform	100 μg/ml	29.66 ± 0.665	168.57 μg
Ethyl acetate	100 μg/ml	28.14 ± 0.528	177.08 μg
Methanol	100 μg/ml	47.51567 ± 0.077	105 μg
Ethanol	100 μg/ml	39.07 ± 0.549	128 μg
Aqueous	100 μg/ml	51.94 ± 0.265	96.26 μg
Standard (acarbose)	100 μg/ml	59.11 ± 0.402	84.58 μg

in perfume making and alcohol industry (http://www.ars-grin.gov/duke; Purabi et al., 2011; Ezhilan and Neelamegam, 2011; Hemalatha and Padmini, 2011; Senthilkumar et al., 2011). However no reports are available on the activities of some identified compounds such as 3-undecene, tridecene, and octadecanal. So present study is expected to help in the identifying antidiabetic activity of selected crude extracts of *Ximenia americana*.

From an extensive literature review it was observed that *Ximenia americana* is widely used as a popular substitute remedy in certain regions of the Africa (Guinea, Ethiopia, Nigeria, Sudan) and in the Brazil. The plant extracts, particularly aqueous and methanolic, showed several biological activities such as antimicrobial, pesticidal, analgesic, antipyretic, anticancer and antitrypanosomal among others (Magassouba et al., 2007; Maikai et al., 2008a; Maikai et al., 2008b; Maikai et al., 2009; Rezanka and Sigler, 2007; Siddaiah et al., 2009; Soro et al., 2009; Voss et al., 2006). Previous studies from our laboratories also showed aqueous and methanolic *Ximenia americana* leaf extracts were potent antioxidant and anti-inflammatory agent (Arun et al., 2015). Hence, the present study also supporting and provides a clear scientific basis that *X. americana* can be a potent source for the novel medicines. Antidiabetic activities of aqueous *X. americana* leaves extract reported for the first time showed its therapeutic potential to be used as a cost effective safe herbal antidiabetic agent (Grover et al., 2002). Accordingly, these results encourage further studies on extracts and identify particular active chemical compounds responsible for the specific biological activity in order to standardize the plant preparation for maximum therapeutic benefit to treat diabetes.

5. Conclusion

Traditional therapeutic plants are frequently used in rural parts, since the availability of extravagant amount of medicinal plants in those areas. Thus, treating diabetes mellitus with herbal derived composites that are accessible and do not necessitate laborious pharmaceutical production seems extremely attractive. This is of great importance to developing countries such as India. The existence of phytochemcials diversities in *X. americana* showed broad spectrum of diverse biological activities and industrial applications such as antioxidant, anti-inflammatory, antiviral, antibacterial, antifungal, dietary nutrient, in perfume making and alcohol industry. These results of GC-MS profile can be used as pharmacognostical tool for the identification of novel drugs from *Ximenia americana.* Based on the results obtained from different *in vitro* anti-diabetic assays, there is significant difference in anti-diabetic activity of different extracts evaluated. Aqueous extract of *X. americana* leaves has shown significant anti-diabetic activity in

Table 7
Percentage of glucose uptake in yeast cells treated with *Ximenia americana* extracts.

Samples	Concentration(μg/ml)	Inhibition (%)	IC50(μg/ml)
Standard	50 μg	47.54 ± 0.183**	52.58 μg
	100 μg	54.67 ± 0.360**	
	150 μg	58.54 ± 0.601**	
	200 μg	62.45 ± 0.824**	
	250 μg	68.06 ± 0.496**	
Chloroform extract	50 μg	30.86 ± 0.316**	261.94 μg
	100 μg	38.26 ± 0.425**	
	150 μg	41.62 ± 0.450**	
	200 μg	45.04 ± 0.349**	
	250 μg	47.72 ± 0.180**	
Ethyl acetate extract	50 μg	22.27 ± 0.398**	314.54 μg
	100 μg	28.90 ± 0.333**	
	150 μg	32.73 ± 0.300**	
	200 μg	34.77 ± 0.560**	
	250 μg	39.74 ± 0.418**	
Methanol extract	50 μg	38.52 ± 0.250**	155.99 μg
	100 μg	44.63 ± 0.537**	
	150 μg	48.08 ± 0.472**	
	200 μg	53.54 ± 0.628**	
	250 μg	58.20 ± 0.309**	
Ethanol extract	50 μg	37.95 ± 0.698*	213.72 μg
	100 μg	39.24 ± 0.894*	
	150 μg	41.60 ± 0.812*	
	200 μg	46.79 ± 0.326*	
	250 μg	50.62 ± 0.870*	
Aqueous extract	50 μg	45.81 ± 0.847**	96.74 μg
	100 μg	51.68 ± 1.409**	
	150 μg	55.73 ± 0.606**	
	200 μg	62.00 ± 0.588**	
	250 μg	67.08 ± 0.499**	

Results are expressed as Mean ± SE (n = 3); * significant at the p < 0.01.
Correlation is significant at the 0.01 level (2-tailed)**
Correlation is significant at the 0.05 level (2-tailed)*

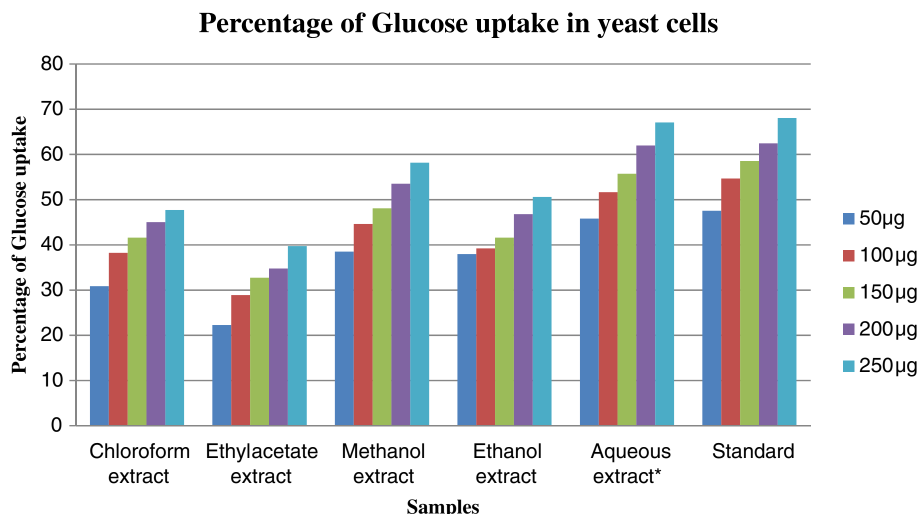

Fig. 7. Percentage of glucose uptake in yeast cells treated with *Ximenia americana* extracts.

both assays compared to other extracts. The present study revealed that aqueous extract exhibited significant *in vitro* anti-diabetic activity. The result also demonstrated that *X. americana* plant can be exploited to discover the bioactive natural products which may serve in the development of new pharmaceuticals. Further, purification of the specific active constituents need to be carried out, that can be used for the discovery of novel drugs to treat diabetes mellitus, a worldwide epidemic disease.

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