**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 extract 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, Trifluorooctacetoxy tetradecene, and Trichlorooctic 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-Tetradecan, and 1-Octyn-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 need for purification, characterization and structural elucidation of bioactive compounds from aqueous extract & also confirms its antidiabetic property by *in vitro* 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 (Kayaraham 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 by 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

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**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 a 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, leprosy, 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 (Ogunleye & Ibitoye, 2003). Many investigations have validated the use of roots in the treatment of leprosy, syphilis, dysentery, and wounds. The stem bark has been reported to have anti-trypanosomal 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 Karnataka University Campus, Dharwad, India in the month of June, 2014. The leaves were identified and authenticated by Dr. Kotesha K., Department of Botany, Karnataka Science College, Dharwad, Karnataka, India. A voucher specimen (N0-01/2016) was deposited at the Department of Botany, Karnataka Science College, Dharwad, Karnataka. Fresh disease free plant material was washed under running tap water, shade dried and...
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 by literature survey and Dr. Duke’s Phytochemical and Ethnobotanical 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
equation:

\[
\text{% inhibition of } \alpha-\text{Amylase} = \left( \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right) \times 100
\]

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

Fig. 3. GC Chromatogram of Methanol 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.

![Fig. 4. GC Chromatogram of Ethanol leaf extract of Ximenia americana.](image)

![Fig. 5. GC Chromatogram of Aqueous leaf extract of Ximenia americana.](image)

### Table 1

| Sl. no. | Compound name     | Molecular formula | Molecular weight | Retention time | Peak area | Similarity index | Compound nature                  | Uses                                      |
|---------|-------------------|-------------------|------------------|----------------|-----------|------------------|----------------------------------|------------------------------------------|
| 1.      | Oleic acid        | C₁₈H₃₄O₂          | 282              | 15.791         | 45.64    | 95%              | Monounsaturated fatty acid       | Anti-oxidant and anti-Breast cancer.     |
| 2.      | n-Hexadecanoic acid| C₁₈H₃₂O₂          | 256              | 14.108         | 35.29    | 93%              | Saturated fatty acid             | Cosmetic, anti-oxidant, anti-bacteria and anti-fungal. |
| 3.      | Non-decanoic acid | C₁₉H₃₈O₂          | 298              | 13.836         | 11.21    | 91%              | Fatty acid                       | Dietary nutrient, anti-inflammatory, Biomarker for Prostate cancer. |
| 4.      | Octadecatrienoic acid | C₁₈H₃₀O₂    | 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                  | C₁₁H₂₂          | 154              | 7.57           | 3.34      | 92%              | –               | –                |
| 2      | Tridecane                   | C₁₃H₂₆          | 182              | 9.987          | 4.65      | 91%              | Fatty acid      | Respiratory irritations. |
| 3      | Trifluoroacetotetradecane   | C₁₄H₂₉F₂O₂      | 310              | 14.050         | 8.24      | 89%              | –               | –                |
| 4      | Trans-1,1-Tetradecanoylaceta| C₁₃H₂₀O₂        | 254              | 12.712         | 5.42      | 87%              | –               | –                |
| 5      | Trichloroacetic acid-3-tridecyl-ester | C₁₃H₂₇Cl₃O | 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           | C₂₄H₄₈O          | 354              | 5.223          | 13.42     | 93%              | Fatty acid alcohol | –                         |
| 2       | Behenyl alcohol          | C₂₂H₄₆O          | 326              | 12.001         | 73.34     | 92%              | Saturated fatty alcohol | Anti-viral agent (Herpes simples virus) |
| 3       | 1-Hexacosanol            | C₂₂H₄₄O          | 382              | 14.041         | 5.21      | 91%              | Saturated fatty alcohol | Wax and Polymer                               |
| 4       | Octadecanal              | C₂₂H₄₄O          | 268              | 15.445         | 0.69      | 90%              | Long chain aldehyde | Pheromone                                |
| 5       | 4-Piperidine propanoic acid | C₉H₁₉CINO      | 351              | 15.727         | 4.27      | 89%              | Long chain aldehyde | Pheromone                                |
| 6       | α-D-mannofuranoside      | C₇H₁₄O           | 194              | 15.783         | 2.63      | 86%              | Carbohydrate     | Thickening agent, plasticizer, and resins. |

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 | C₂₀H₃₄O₂        | 306              | 14.048         | 30.18     | 92%              | Polyunsaturated fatty acid | Prostaglandins                           |
| 2       | 7-Tetradecenal              | C₁₀H₂₀O₂         | 210              | 15.737         | 69.02     | 89%              | Alcohol          | Alcohol Industry                          |
| 3       | 1-Octyn-3-OI-4-ETHY         | C₁₀H₁₄O         | 154              | 15.783         | 0.80      | 81%              | Ester            | Perfume flavor and Food additives         |
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, Trifluoroacetoeoxy tetracene, Trans-11Tetradecenylacetate and Trichloroacetic acid–3-tridecyl-ester in ethyl acetate extract; where in 1-Tetrasacanol, Behenyl alcohol, 1-Hexacosanol, Octadecanal, 4-Piperidine propanoic acid and α –D-mannofuranoside in methanol extract; 8,11,14-Eicosatrienioic acid, 7-Tetradecanal and 1-Octyn-3-Ol-4-Ethyl in ethanol extract; aqueous extract showed the presence of 9,12-Octadecadionioic 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 (Agoramoorothy et al., 2007); 9,12-Octadecadionioic acid was reported to have anti-inflammatory, antibacterial, hypocholesterolemic and hepatoprotective activity (Sermakkan and 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, anti-tumoral, antibacterial, antifungal, dietary nutrient, anti-inflammatory, antiviral, antibacterial, antifungal, dietary nutrient,

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### Table 5

| Sl. no. | Compound name | Molecular formula | Molecular weight | Retention time | Peak area | Similarity index | Compound nature | Uses |
|---------|---------------|-------------------|------------------|---------------|----------|----------------|----------------|------|
| 1.      | 9,12-Octadecandionioic acid | C₁₉H₃₂O₂ | 280 | 21.658 | 100 | 86% | Poly-unsaturated fatty acid | Anti-oxidant, surfactant and Oil paints |

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**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.
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; Siddiaiah 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 (Arum 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 particularly 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 phytochemicals 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 6**

| Samples          | Concentration (µg/ml) | Inhibition [%] | IC50 (µg/ml) |
|------------------|-----------------------|---------------|--------------|
| Chloroform       | 100                    | 29.66 ± 0.665 | 168.57 µg    |
| Ethyl acetate    | 100                    | 28.14 ± 0.528 | 177.08 µg    |
| Methanol         | 100                    | 47.51567 ± 0.777 | 105 µg    |
| Ethanol          | 100                    | 39.07 ± 0.549 | 128 µg       |
| Aqueous          | 100                    | 51.94 ± 0.245 | 96.26 µg     |
| Standard (acarbose) | 100                | 59.11 ± 0.402 | 94.58 µg     |

**Table 7**

| Samples          | Concentration (µg/ml) | Inhibition [%] | IC50 (µg/ml) |
|------------------|-----------------------|---------------|--------------|
| Standard         | 50                     | 47.54 ± 0.183 | 52.58 µg     |
| Methanol extract | 50                     | 38.26 ± 0.425 | 261.94 µg    |
| Ethanol extract  | 50                     | 22.27 ± 0.398 | 314.54 µg    |
| Aqueous extract  | 50                     | 39.74 ± 0.418 | 155.99 µg    |

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