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Gayathri S
Sathyabama Institute of Science and Technology, OMR Road, Chennai, Tamil Nadu, India

Sivaraj C
Armats Biotek Training and Research Institute, Guindy, Chennai, Tamil Nadu, India

Sangeetha ST
Sathyabama Institute of Science and Technology, OMR Road, Chennai, Tamil Nadu, India

Arumugam P
Sathyabama Institute of Science and Technology, OMR Road, Chennai, Tamil Nadu, India

A Manimaran
P.G and Research Department of Advanced Zoology and Biotechnology, Government Arts College for Men, Nandanam, Chennai, Tamil Nadu, India

Correspondence
A Manimaran
P.G and Research Department of Advanced Zoology and Biotechnology, Government Arts College for Men, Nandanam, Chennai, Tamil Nadu, India

Evaluation of antioxidant, antibacterial, alpha amylase enzyme inhibition activities and GC-MS analysis of leaves extract of *Gymnema sylvestre* L.

Gayathri S, Sivaraj C, Sangeetha ST, Arumugam P and A Manimaran

Abstract
Gymnema sylvestre is an important medicinal plant which belongs to the family Apocynaceae, commonly used herb in Ayurveda. The common name is gurmar, meaning of "sugar destroying," was given because of the plant has antisacharogenic property (suppresses the taste of sugar). The aim of the present study was to evaluate the antioxidant, antibacterial activities and to identify the bioactive compounds by GC-MS analysis of methanol leaves extract of *G. sylvestre*. Different antioxidant assays were carried out for evaluating antioxidant activity. The maximum DPPH radical scavenging activity was 54.08±0.15% at 120 µg/mL concentration with the IC_{50} of 104.66 µg/mL concentration. The maximum DPPH radical scavenging activity was 54.08±0.15% at 120 µg/mL concentration with the IC_{50} of 104.66 µg/mL concentration. The maximum superoxide radical ($O_2^{\cdot-}$) scavenging activity was 62.48±0.20% at 120 µg/mL concentration with the IC_{50} of 72.93 µg/mL concentration. The maximum Fe^{3+} reduction and Mo^{6+} reduction were 83.11±0.17% and 62.03±0.36% at 120 µg/mL concentration with the RC_{50} of 43.79 µg/mL and 75.14 µg/mL concentration respectively. The maximum α-amylase enzyme inhibition was 70.86±0.94% at 60 µg/mL concentration with the IC_{50} of 75.40 µg/mL concentration.

Keywords: *G. sylvestre*, antibacterial, antioxidant activity, DPPH**,** GC-MS

1. Introduction
G. sylvestre is a perennial, woody climber belonging to family Asclepiadaceae or the "milk weed" family [1]. *G. sylvestre* commonly known as sakkarai kolli, has been broadly used in indigenous systems of Indian medicine due to its numerous therapeutic properties [2]. *G. sylvestre* is native to India, found in tropical and subtropical regions, but widely well distributed in the southern part of China, tropical Africa, Malaysia, and Sri Lanka [3]. The leaves are opposite, usually elliptic, elongate oval (1.25–2.0 inch × 0.5–1.25 inch) shaped and have soft hairs on the upper surface, inflorescence is lateral umbel in cymes; follicles are terete and lanceolate, up to 3 inches in height. Flowers are greenish white in colour. Corolla is pale yellow in colour, valvate, campanulate with single corona with 5 fleshy scales. The calyx-lobes are long, ovate, obtuse, and pubescent. Carpels-2, unilocular, ovules locules may be present, anther connective produced into a membranous tip [4, 5].

1.1 Taxonomy
Kingdom: Plantae
Phylum: Tracheophyta
Subphylum: Angiosperms
Class: Eudicots
Order: Gentianales
Family: Apocynaceae
Genus: *Gymnema*
Species: *sylvestre*
Binomial name: *Gymnema sylvestre*

Fig 1: *Gymnema sylvestre*

2. Materials and Methods

2.1 Collection of leaves and preparation of extract

The leaves of *G. sylvestre* were collected from the market at Mylapore, Chennai, Tamil Nadu, India. The leaves were washed, shade dried for 10 d and powdered in mechanical blender. About 10 g of leaves powder was soaked in methanol for 72 h. The greenish supernatant liquid was filtered by filter paper and condensed in a hot plate at 50°C, which yields gummy extract.

2.2 Qualitative phytochemical analysis

The methanol leaves extract of *G. syvestre* was subjected to different classes of phytoconstituents, using specific reagents and following standard methods [6].

2.3 Estimation of total phenols

Folin-Ciocalteau reagent method was used to determine the total phenolic compounds [7] with slight modifications. One hundred µL of methanol leaves extract (1mg/mL) was mixed with 900 µL of methanol and 1 mL of Folin Ciocalteau reagent (1:10 diluted with distilled water). After 5 min, 1 mL of 20% (w/v) of Na_2CO_3 solution was added. The mixture was then allowed to stand for 30 min incubation in dark at room temperature. The absorbance was measured at 765 nm in UV-Vis spectrophotometer. The total phenolic content was expressed in terms of gallic acid equivalent (µg/mg of extract), which is a common reference compound.

2.4 Estimation of total flavonoids

The total flavonoid content was determined using aluminium chloride reagent method with slight modification [8]. Five hundred µL of methanol leaves extract (1mg/mL) was mixed with 500 µL of methanol and 500 µL of 5% (w/v) sodium nitrite solution followed by 500 µL of 10% (w/v) aluminium chloride solution was added and incubated for 5 min at room temperature. Then 1 mL of 1 M NaOH solution was added and the total volume was made up to 5 mL with distilled water. Absorbance was measured at 510 nm in UV-Vis spectrophotometer. The result was expressed as (µg/mg of extract) quercetin equivalent.

2.5 *In vitro* antioxidant assays

2.5.1 DPPH˙ radical scavenging assay

The antioxidant activity of methanol leaves extract of *G. sylvestre* was measured on the basis of stable DPPH free radical reduction method [9]. One mL of 0.1 mM DPPH solution in methanol was mixed with 1 mL of various concentrations (20-120 µg/mL) of leaves extract. The mixture was then allowed to stand for 30 min incubation in dark. One mL methanol and 1 mL DPPH solution was used as the control. The decrease in absorbance was measured using UV-Vis Spectrophotometer at 517 nm. Ascorbic acid was used as the standard reference. The percentage of inhibition was calculated as:

$$\% \text{ of DPPH}^{\cdot} \text{ radical inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}}\right] \times 100$$

2.5.2 Superoxide radical ($O_2^{\bullet-}$) scavenging assay

Superoxide radical scavenging activity was carried out by the method of Ravishankara et al [10]. Different concentrations of leaves extract (20-120 µg/mL) of *G. sylvestre* was mixed with 50 mM of phosphate buffer (pH 7.8), 1.5 mM of riboflavin, 12 mM of EDTA and 50 mM of NBT solutions and added in that sequence. The reaction was started by illuminating the reaction mixture for 15 min. After illumination, the absorbance was measured at 590 nm in UV-Vis Spectrophotometer. Ascorbic acid was used as standard reference. The percentage of inhibition was calculated as:

$$\% \text{ of superoxide radical inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}}\right] \times 100$$

2.5.2 $ABTS^{\cdot+}$ radical cation scavenging assay

The antioxidant capacity was estimated in terms of the $ABTS^{\bullet+}$ radical cation scavenging activity [11]. $ABTS^{\bullet+}$ was obtained by reacting 7 mM ABTS solution in 5 mM of phosphate-buffered saline (pH 7.4) with 2.45 mM potassium persulfate and the mixture was left to stand in dark at room temperature for 12-16 h before use. The ABTS solution (stable for 2 days) was diluted with 5 mM phosphate-buffered saline (pH 7.4) till to reach an absorbance of 0.70±0.02 at 734 nm. To the various concentrations (20-120µg/mL) of methanol leaves extract of *G. sylvestre*, 500 µL of diluted $ABTS^{\bullet+}$ solution was added. The absorbance was measured after 10 min incubation at 734 nm. Ascorbic acid was used as the standard reference. The $ABTS^{\bullet+}$ radical cation scavenging activity was expressed as:

$$\% \text{ of } ABTS^{\cdot+} \text{ radical cation inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}}\right] \times 100$$

2.5.3 Ferric (Fe^{3+}) reducing power assay

The reducing power of methanol leaves extract of *G. sylvestre* was determined by Fe^{3+} reduction method with slight modification [12]. One mL of leaves extract of different concentrations (20 - 120 µg/mL) was mixed with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1 % w/v). The mixtures were then incubated at 50°C in water bath for 30 min. One mL of trichloroacetic acid (10 % w/v) was added to each mixture. Then 1 mL of freshly prepared $FeCl_3$ (0.1% w/v) solution was added and the absorbance was measured at 700 nm in UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of reduction was calculated as:

$$\% \text{ of } Fe^{3+} \text{ reduction} = \left[\frac{\text{Sample} - \text{Control}}{\text{Sample}}\right] \times 100$$

2.5.4 Phosphomolybdenum reduction assay

The antioxidant capacity of methanol leaves extract of *G. sylvestre* was assessed by Mo^{6+} reduction method [13]. The leaves extract with concentrations ranging from 20 to 120 µg/mL was combined with 1 mL of reagent solution containing ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulphuric acid (600 mM). The reaction mixture was incubated in water bath at 95°C for 90 min. The absorbance of the coloured complex was measured at 695 nm in UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of reduction was calculated as:

$$\% \text{ of phosphomolybdenum reduction} = \left[\frac{\text{Sample} - \text{Control}}{\text{Sample}}\right] \times 100$$

2.6 Antidiabetic activity

2.6.1 Alpha amylase enzyme inhibition assay

α- amylase enzyme inhibition assay was carried out based on the starch-iodine test [14]. The total assay mixture was composed of various concentration (20-120 µg/mL) of leaves extract of *G. sylvestre*, 10 µL of alpha amylase enzyme prepared in 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride) and was incubated at 37°C for 10 min. Then soluble starch (1%, w/v) was added to each reaction set and incubated at 37°C for 60 min. One hundred µL of 1 M HCl was added to stop the enzymatic reaction and followed by 200 µL of iodine reagent (5 mM I_2 and 5 mM KI) was added. The colour change was noted and the absorbance was read at 595 nm. The control reaction representing 100% enzyme activity did not contain any plant extract. A dark-blue colour indicates the presence of starch; a yellow colour indicates the absence of starch, while a brownish colour indicates partially degraded starch in the reaction mixture. In the presence of inhibitors from the extracts, the starch added to the enzyme assay mixture is not degraded and gives a dark-blue colour complex, whereas no colour complex is developed in the absence of the inhibitor, indicating that starch is completely hydrolyzed by α-amylase.

$$\% \text{ of } \alpha\text{- amylase enzyme inhibition} = \left[\frac{\text{Sample} - \text{Control}}{\text{Sample}} \right] \times 100$$

2.7 Antibacterial activity

2.7.1 Microbial strains

The microorganisms of Gram positive strains such as *Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus luteus* as well as Gram negative strains such as *Escherichia coli*, *Shigella flexneri* and *Proteus vulgaris* were used for the evaluation of antibacterial activity.

2.7.2 Reference and control

Tetracycline was used as the standard for bacteria. The controls consist of solidifying agar onto which was solvent, and the extract was soluble in it.

2.7.3 Aseptic conditions

The aseptic chamber which consist of a wooden box (1.3m x 1.6m x 0.6m) with a door, was cleaned with 70% ethanol and irradiated with short wave UV light (from lamp).

2.7.4 Nutrient broth agar medium

Nutrient broth agar medium was prepared (peptone-5 g; yeast extract-3 g; NaCl-5 g; distilled water-1000 mL; pH-7.0±0.2; Agar-20 g) according to the standard method and required amount was weighed, suspended in required volume of 200 mL distilled water in a 500 mL conical flask, stirred and then autoclaved at 15 lbs and at 121ºC for 15 min [15]. The hot medium was poured in sterile petriplates which were kept in the aseptic Laminar chamber. The medium was allowed to solidify for 15 min.

2.7.5 Agar well-diffusion method

The potential antibacterial activity of leaves extract was analyzed by agar well diffusion method [16]. The solidified nutrient agar in the petriplates was inoculated by dispensing the inoculums using sterilized cotton swabs and spread evenly onto the solidified agar medium. Five wells were created in each plate with the help of a sterile well-borer of 8 mm diameter. The extract was then poured into each well to get desirable concentrations. Tetracycline was used as the standard with the concentration of 25 µg. All the plates containing sample loaded wells were incubated for 24 h at 37°C. After the incubation period, zone of inhibition in each plate, for each concentration of extract and standard were measured by calculating the diameter of zone of inhibition.

2.8 Gas chromatography–Mass Spectrometry (GC–MS)

For GC-MS analysis, the samples were injected into a HP-5 column (30 m X 0.25 mm i.d with 0.25 µm film thickness), Agilent technologies 6890 N JEOL GC Mate II GC-MS model. Following chromatographic conditions were used: Helium as carrier gas, flow rate of 1 mL/min; and the injector was operated at 200°C and column oven temperature was programmed as 50-250°C at a rate of 10°C/min injection mode. Following MS conditions were used: ionization voltage of 70 eV; ion source temperature of 250°C; interface temperature of 250°C; mass range of 50-600 mass units.

2.8.1 Identification of components

The database of National Institute Standard and Technology (NIST) having more than 62,000 patterns was used for the interpretation on mass spectrum of GC-MS. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library [17].

2.9 Results and Discussion

2.9.1 Total phenols and flavonoids

Phenolic compounds are predominantly distributed in plants and they have gained much attention, due to their antioxidant activity and free radical scavenging ability with potential benefits for human health [18]. Natural antioxidants available in medicinal plants have been alternative source for synthetic antioxidants. Free radicals are formed naturally in the body and play an important role in many normal cellular processes. At high concentrations, however, free radicals can be hazardous to the body and damage all major components of cells, including DNA, proteins, and cell membranes. The damage to cells caused by free radicals, especially the damage to DNA, may play a role in the development of cancer and other health conditions. Antioxidants such as phenols and flavonoids offer resistance against oxidative stress by preventing free radicals which are damaging biomolecules such as proteins, DNA, and lipids, thus prevent disease progression [19]. Phenolic compounds such as flavonoids, phenolics acid, and tannins possess diverse biological activities including antiinflammatory, anticarcinogenic, and antiatherosclerotic activities. The phenols and flavonoids were quantified in the methanol extract of *G. sylvestre* seemed to be responsible for antioxidant activity. The total phenolic content was 369.17 µg/mg of GAE and the total flavonoid content was 20.44 µg/mg of QE in the extract.

2.9.2 DPPH˙ radical scavenging assay

DPPH˙ (1,1-Diphenyl-2-picrylhydrazyl) is a stable nitrogen-centered free radical which has an unpaired valence electron at one atom of nitrogen bridge. Scavenging of DPPH free radical is one of the popular antioxidant assays. DPPH radical scavenging assay is a decolorization assay that will measure the capacity of antioxidants to directly scavenge DPPH˙ radicals by monitoring its absorbance using spectrophotometer at wavelength of 517 nm [20]. The DPPH assay provided rapid and an easy way to evaluate the antioxidant activity of most of the plant extracts. The

methanol leaves extract of *G. sylvestre* to scavenge free radicals was assessed by using DPPH· radical as the substrate, which measures the hydrogen or electron donating ability of leaves extract. The leaves extract of *G. sylvestre* reducing the stable purple colour DPPH (1,1-diphenyl-2- picrylhydrazyl) free radical to the yellow coloured 1,1-diphenyl-2-picrylhydrazine and the reduction capacity increases with increasing concentration of the extract. The maximum DPPH· radical scavenging activity was 54.08±0.15% at 120 µg/mL concentration. The IC_{50} was 104.64 µg/mL concentration and was compared with standard ascorbic acid (IC_{50} = 6.31 µg/mL concentration).

Table 1: DPPH· radical and superoxide ($O_2^{\cdot-}$) radical scavenging assay of methanol leaves extract of *G. Sylvestre*

S. No	Concentration (µg/mL)	Inhibition / %	
		DPPH· radical at 517 nm	Superoxide ($O_2^{\cdot-}$) radical at 590 nm
1	20	23.87±0.32	21.66±0.36
2	40	29.79±0.67	29.57±0.49
3	60	39.08±0.51	39.36±0.19
4	80	44.29±1.03	54.84±0.47
5	100	47.77±0.92	56.49±0.12
6	120	54.08±0.15	62.48±0.20

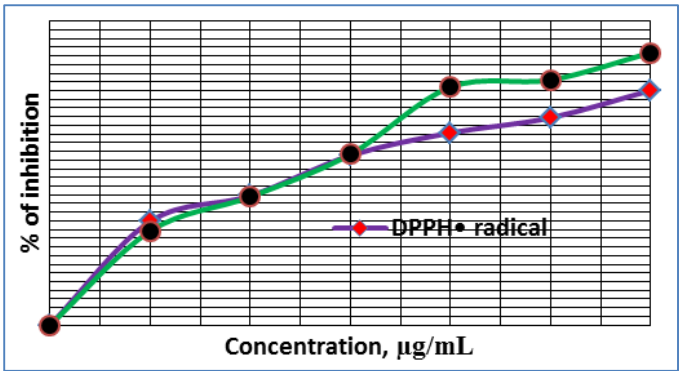


Fig 2: DPPH· radical and superoxide ($O_2^{\cdot-}$) radical scavenging assay of methanol leaves extract of *G. Sylvestre*

2.9.3 Superoxide ($O_2^{\cdot-}$) radical scavenging assay

Superoxide anion is also very harmful to cellular components and their effects can be magnified because it produces other kinds of free radicals and oxidizing agents [21]. Flavonoids are effective antioxidants, mainly because they scavenge superoxide anions. Superoxide anions derived from dissolved oxygen by the riboflavin-light-NBT system will reduce NBT in this system. In this method, superoxide anion reduces the yellow dye (NBT^{2+}) to blue formazan, which is measured at 590 nm in UV-Vis spectrophotometer. Antioxidants are able to inhibit the blue NBT formation and the decrease of absorbance with antioxidants indicates the consumption of superoxide anion in the reaction mixture. The maximum superoxide radical scavenging activity of *G. sylvestre* was 62.48±0.20% at 120 µg/mL concentration and the IC_{50} was 72.93 µg/mL concentration. It was compared with the standard of ascorbic acid (IC_{50} = 9.65 µg/mL concentration).

2.9.4 $ABTS^{\bullet+}$ radical cation scavenging assay

$ABTS^{\bullet+}$ is a blue-green chromophore produced by the reaction between ABTS and potassium persulfate. The blue-green chromophore radical cation gets reduced while reacting an antioxidant in the leaves extract of *G. sylvestre* and the remaining radical cation concentration was quantified with the loss of colour [22]. The antioxidant reduces $ABTS^{\bullet+}$ to ABTS and decolorize the blue-green chromophore. The maximum ABTS•+ radical cation scavenging activity was 95.26±0.55% at 30 µg/mL concentration with the IC_{50} of 13.94 µg/mL concentration and was compared with standard ascorbic acid (IC_{50} = 4.21 µg/mL concentration).

Table 2: $ABTS^{\cdot+}$ radical cation scavenging assay of methanol leaves extract of *G. sylvestre*

S. No	Concentration (µg/mL)	Inhibition / %
		$ABTS^{\cdot+}$ at 734 nm
1	5	19.15±0.58
2	10	39.69±0.79
3	15	53.76±0.82
4	20	65.02±0.28
5	25	74.15±0.33
6	30	95.26±0.55

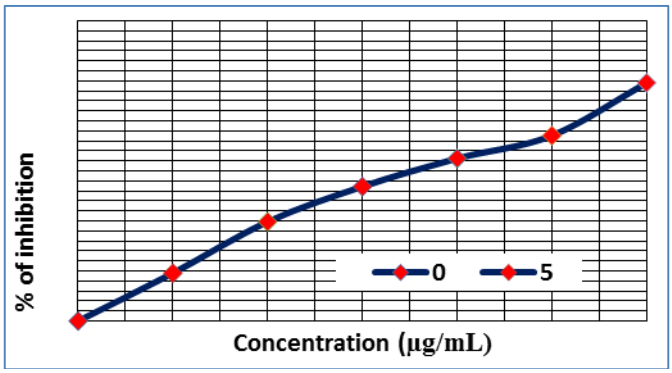


Fig 3: $ABTS^{\bullet+}$ radical cation scavenging assay of methanol leaves extract of *G. sylvestre*

2.9.5 Ferric (Fe^{3+}) reducing power assay

The reducing power assay was carried out by the reduction of Fe^{3+} to Fe^{2+} by the methanol leaves extract of *G. slvestre* and the subsequent formation of ferro-ferric complex. Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action [23]. The reducing ability of a compound generally depends on the presence of reductones (antioxidants), which exert the antioxidant activity by breaking the free radical chain by donating a hydrogen atom or by neutralizing the free radicals by donating an electron and become lone pair of electrons instead of odd electron. The reduction ability increases with increase in concentration of the extract. The maximum Fe^{3+} reduction was 83.11±0.17% at 120 µg/mL concentration with the RC_{50} of 45.78 µg/mL concentration and was compared with the standard ascorbic acid (RC_{50} =7.72 µg/mL).

2.9.6 Phosphomolybdenum reduction assay

Metal-Catalyzed Oxidation (MCO) systems catalyze the reduction reaction, which alters the nature of proteins at the metal-binding site and cause DNA and protein damage [24]. The total antioxidant activity of methanol leaves extract of *G. slvestre* was measured by phophomolybdenum reduction method which is based on the reduction of Mo (VI) to Mo(V) by the formation of green phosphate/Mo (V) complex at acidic pH, with a maximum absorption at 695 nm. The maximum phosphomolybdenum reduction was 62.03±0.36% at 120 µg/mL concentration with the RC_{50} of 86.31 µg/mL concentration. It was compared with the standard ascorbic acid (RC_{50} =6.34 µg/mL).

Table 3: Fe^{3+} reducing power and phophomolybdenum reduction assay of methanol leaves extract of *G. sylvestre*

S. No.	Concentration (µg/mL)	Reduction / %	
		Fe^{3+} reduction at 700 nm	Phosphomolybdenum reduction at 695 nm
1	20	21.85±.0.22	12.80±0.29
2	40	45.67±0.57	32.76±0.57
3	60	62.29±0.63	46.62±0.65
4	80	68.78±0.43	53.23±0.67
5	100	73.64±0.65	57.92±0.58
6	120	83.11±0.17	62.03±0.36

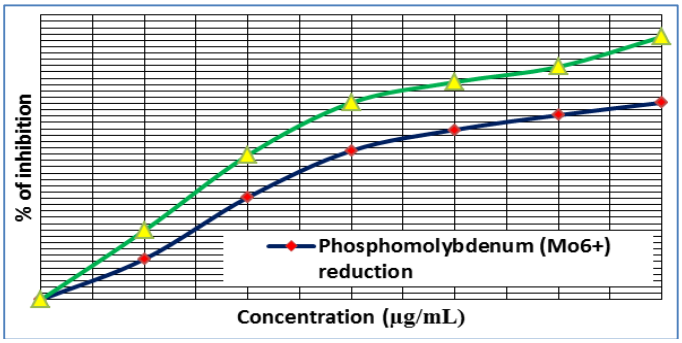


Fig 4: Fe^{3+} reducing power and phosphomolybdenum reduction assay of methanol leaves extract of *G. sylvestre*

2.9.7 Alpha amylase enzyme inhibition assay

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia and its type II is the major form of diabetes. The management of the blood glucose level is a critical strategy in the control of diabetes complications. The inhibition of carbohydrate hydrolyzing enzymes such as α-amylase can be an important strategy to lower postprandial blood glucose levels. Such synthetic inhibitors of diabetes are known to be associated with various gastrointestinal side effects. Therefore, it is the need of time to identify and explore the amylase inhibitors from natural sources having fewer side effects. One therapeutic approach which may prove to be beneficial for treatment of diabetes is to decrease the post-prandial hyperglycemia. This can be achieved by retarding the absorption of glucose through the inhibition of the carbohydrate hydrolyzing enzymes in the digestive tract. The α-amylase and α- glucosidase enzymes are responsible for the breakdown of oligo and/or disaccharide to monosaccharides. Inhibitors of these enzymes delay carbohydrate digestion and prolong overall carbohydrate digestion time causing a marked decrease in the rate of glucose absorption thereby blunting the post prandial plasma glucose rise [25]. The maximum α-amylase enzyme inhibition was 70.86±0.94% at 120 µg/mL concentration and the IC_{50} was 75.40 µg/mL concentration.

Table 4: α-Amylase enzyme inhibition assay of methanol leaves extract of *G. sylvestre*

S. No.	Concentration (µg/mL)	Inhibition / %
		α-amylase enzyme inhibition at 595 nm
1	20	08.92±0.27
2	40	11.61±0.77
3	60	22.16±0.45
4	80	53.05±0.15
5	100	60.22±0.97
6	120	70.86±0.94

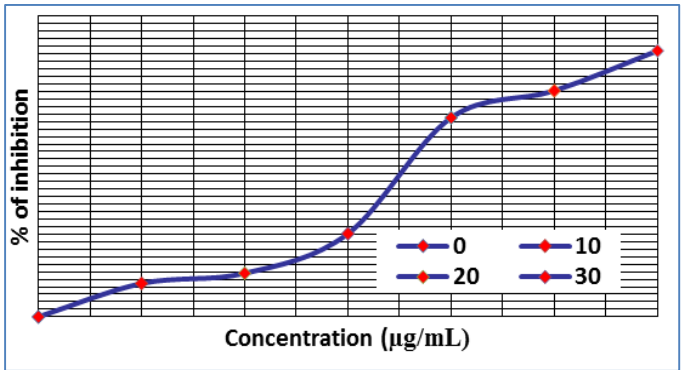


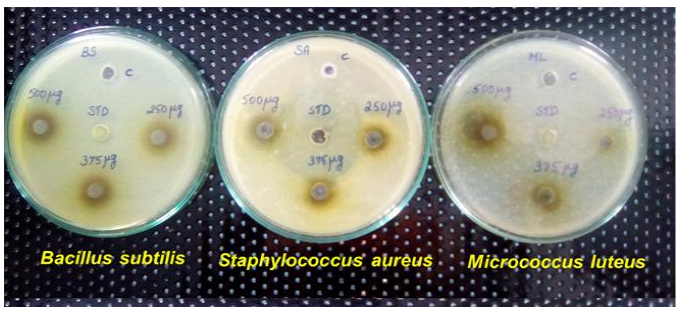
Fig 5: α-amylase enzyme inhibition assay of methanol leaves extract of *G. sylvestre*

2.9.7 Antibacterial activity by agar well diffusion method

The antibacterial activity was carried out for methanol leaves extract of *G. sylvestre* against Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus and Micrococcus luteus*) and Gram-negative bacteria *(Escherichia coli, Shigella flexneri and Proteus vulgaris*). The antibacterial sensitivity of the crude extract and their potency were assessed quantitatively by measuring the diameter of clear zone in cultures in petriplates. The antibacterial activity of the extract could be correlated to the presence of secondary metabolites such as alkaloids, terpenoids and phenolic compounds that adversely affect the growth and metabolism of microbes. The maximum zone of inhibition of 14 mm for *Micrococcus luteus* and of 14 mm for *Proteus vulgaris* were showed at 500 µg/mL concentration of the extract. The molecular components and morphology of membranes from Gram-positive bacteria are fundamentally different from those of Gram-negative bacteria. Gram-negative bacteria are surrounded by two membranes, the cytoplasmic cell membrane and the outer membrane. The outer monolayer of the membrane contains lipopolysaccharide (LPS) as the major lipid component, a lipid species unique to Gram-negative bacteria [26].

Table 5: Antibacterial activity of methanol leaves extract of *G. sylvestre*

Bacterial pathogens	Zone of inhibition (mm)			
	Standard (Tetracycline)	250 µg/mL	375 µg/mL	500 µg/mL
Bacillus subtilis	11	11	12	13
Staphylococcus aureus	12	10	11	12
Micrococcus luteus	11	-	11	14
Escherichia coli	11	11	12	13
Shigella flexneri	11	10	11	12
Proteus vulgaris	11	12	13	14



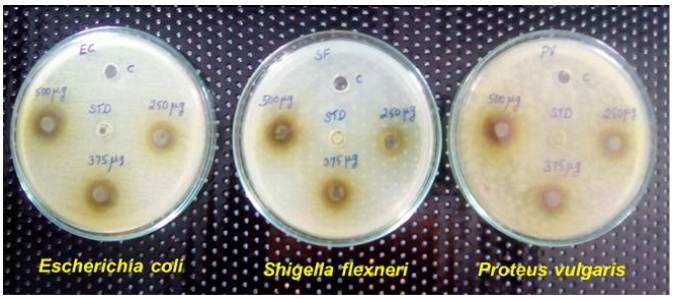


Fig 6: Antibacterial activity of methanol leaves extract of *G. sylvestre*

2.10 GC-MS analysis

GC-MS analysis was carried out for methanol leaves extract of *G. Sylvestre* and the antibacterial compound [27] globulol as well as antioxidant compound phytol and dasycarpidan-1-methanol acetate(ester)and anti-inflammatory compound[28] were eluted (Fig 7 and Table 6) and recorded.

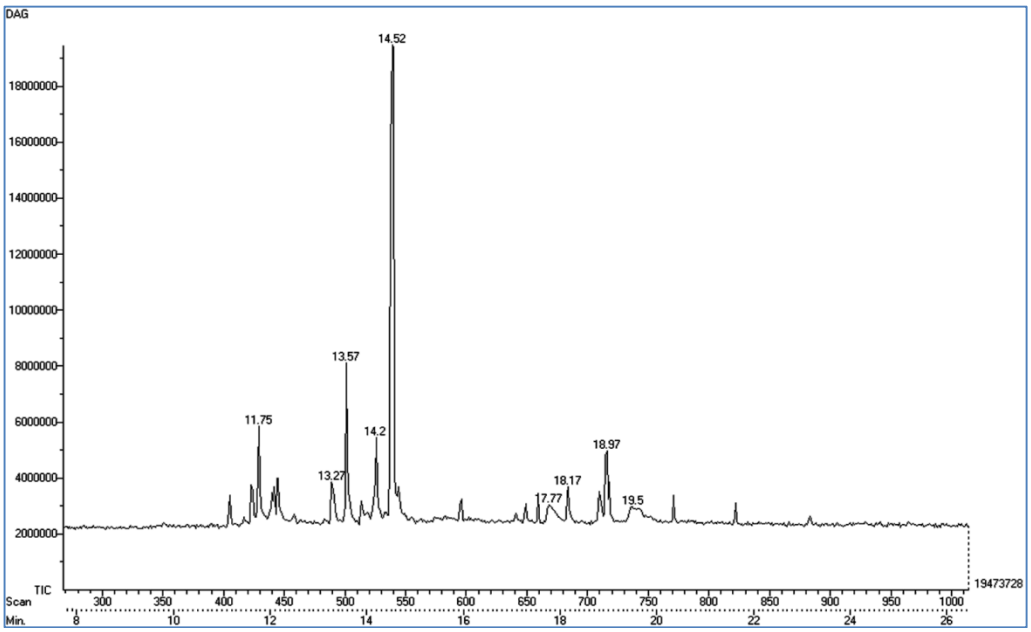
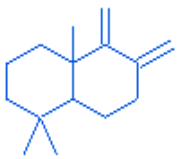
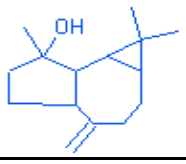
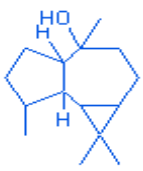
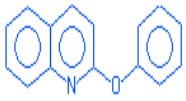
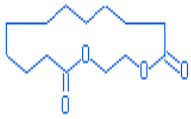
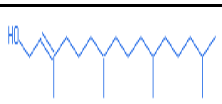
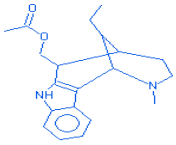


Fig 7: Gas chromatogram of methanol leaves extract of *G. sylvestre*

Table 6: Active compounds eluted and identified in methanol leaves extract of *G. sylvestre* by GC-MS analysis

S. No	RT	Name	Structure	Mol. Wt g/mol	Mol. formula
1.	11.75	1,1,4a-trimethy-5,6 dimethylenedecahydro naphthalene		204.08	$C_{13}H_{20}O$
2.	13.27	Spathulenol		220.13	$C_{15}H_{24}O$
3.	13.57	Globulol		222.11	$C_{25}H_{26}O$
4.	14.15	Quinoline,2-phenoxy		220.98	$C_{15}H_{11}NO$

5.	14.52	Cyclongifolene oxide dehydro		218.08	$C_6H_{10}O$
6.	17.77	1,4-dioxyacylcohexadecane 5,16-dione		336.64	$C_{14}H_{24}O_4$
7.	18.17	Hexadeca-2,6,10,14-tetraen-1-ol,3,7,11,16-tetramethyl		290.17	$C_{20}H_{34}O$
8.	18.97	phytol		296.16	$C_{20}H_{40}O$
9.	19.50	Dasycarpidan-1-methanol acetate(ester)		326.98	$C_{20}H_{26}N_2O_2$

2.10 Conclusion

Based on the antioxidant and α-amylase enzyme inhibition activities of methanol leaves extract of *G. Sylvestre* showed better antihyperglycemic activity than antioxidant activity. Natural products and their related moieties have historically been incredible as a source of therapeutic agents. Natural products in the pharmaceutical industry have reduced, owing to issues such as the lack of compatibility of traditional natural product extract libraries with high-throughput screening. It has long been recognized that natural product structures have the characteristics of high chemical diversity, biochemical specificity and other molecular properties that make them favourable as lead structures for drug discovery, and which serve to differentiate them from libraries of synthetic and combinatorial compounds. The essential breakthroughs in separation and structure determination technologies have lowered the hurdles inherent in screening mixtures of structurally complex molecules. The confluence of these technologies with advances in genomics, metabolic engineering and chemical synthesis offer the new method along with the technologies to explore the remarkable chemical diversity of nature's 'small molecules' in the pursuance for new drugs.

3. References

1. Saneja A, Sharma C, Aneja KR, Pahwa R. Gurmar (*Gymnema sylvestre*): A review. Der Pharmacia Letter. 2010; 2:275-284.
2. Patil PM, Chaudhari PD, Duragkar NJ, Katolkar PP. Formulation of anti-diabetic liquid preparation of *Gymnema sylvestre* and qualitative estimated by TLC. Asian Journal of Pharmaceutical and Clinical Research. 2012; 5(1):16-19.
3. Singh VK, Umar S, Ansari SA, Iqbal M. *Gymnema sylvestre* for diabetics. Journal of Herbs, Spices and Medicinal Plants. 2008; 14:88-106.
4. Gurav S, Gulkari V, Duragkar N, and Patil A. A. Systemic review: pharmacognosy, phytochemistry, pharmacology and clinical applications of *Gymnema sylvestre* R Br. Pharmacognosy Reviews. 2007; 1:338-343.
5. Potawale SE, Shinde VM, Anandi L, Borade S, Dhalawat H, Deshmukh RS. *Gymnema sylvestre*: A comprehensive review. Pharmacology online. 2008; 2:144-157.
6. Raaman N. Phytochemical techniques. New India Publishing Agency, New Delhi, 2006, 306.
7. Zhinshen, J, Mengcheng T Jianming W. The determination of flavonoid content in mulberry and their scavenging effects in superoxide radicals. Food Chem. 1999; 64:555-559.
8. Ahmed D, Fatima K, Saeed R. Analysis of phenolic and flavonoid contents, and the anti-oxidative potential and lipid peroxidation inhibitory activity of methanolic extract of *Carissa opaca* roots and its fractions in different solvents. Antioxidants. 2014; 3:671-683.
9. Khalaf NA, Shakya AK, Al-othman A, El-agbar Z, Farah H. Antioxidant activity of some common plant. Turk J Biol. 2008; 32:51-5.
10. Bagul MS, Ravishankara MN, Padh H, Rajani M. Phytochemical evaluation and free radical scavenging properties of rhizome of *Bergenia ciliata* (Haw) Sternb: Forma ligulata. Yeo. J Nat Rem. 2003; 3:83-9.
11. Arnao MB, Cano A, Acosta M. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem. 2001; 73:239-44.
12. Oyaizu M. Studies on products of browning reaction: antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. 1986; 44: 307-315.
13. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical Biochemistry 1999; 269: 337-341.
14. Hossan SJ, El-Sayed M, Aoshima H. Antioxidative and anti α-amylase activities of four wild plants consumed by nomads in Egypt. Orient Pharm Exp Med. 2009; 9(3):217-224.
15. Kubo I, Muroi H, Himejima M. Antimicrobial activity of green tea flavor components and their combination effects. Journal of Agric Food Chem. 2002; 40:245-248.

16. Obeidat M, Shatnawi M, Al-alawi E, Al-Zu`bi H, Al-Dmoor M, Al-Qudah J, El-Qudah, Otri I. Antimicrobial Activity of Crude Extracts of Some Plant Leaves. Research Journal of Microbiology. 2012; 7:59-67.
17. Ravisankar N, Sivaraj C, Seeni S, Jerrine joseph, Raaman N. GC-MS Analysis and anticancer activity of methanol extract of leaves of *Hypericum hookerianum* wight & arn. Int J Pharm Pharm Sci. 2014; 6:515-519.
18. Ross JA, Kasum CM. Dietary flavonoids: Bioavailability, metabolic effects, and safety. Annu. Rev. Nutr. 2002; 22:19-34.
19. Braugghler JM, Duncan CA, Chase LR. The involvement of iron in lipid peroxidation: importance of ferrous to ferric ratio in initiation. J. Biol. Chem. 1986; 61:102-182.
20. 20 Awika M, Rooney LW, Wu X, Prior RL, Cisneros-Zevallos L. Screening methods to measure antioxidant activity of Sorghum (*Sorghum ialmatei*) and Sorghum product. Journal of Agricultural and Food Chemistry. 2003; 51:6657-62.
21. Wickens AP. Aging and the free radical theory, Respiratory Physiology. 2001; 128:379-391.
22. Miller DD. Mineral. In: Fennema, O.R. (Ed.), Food Chemistry 1996; Marcel Deckker, New York, 618-649.
23. Yıldırım A, Mavi A, Kara AA. Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. J. Agric. Food Chem. 2001; 49:4083-4089.
24. Stadtman ER. Metal ion-catalyzed oxidation of proteins: Biochemical mechanism and biological consequences. Free Radical Biology and Medicine. 1990; 9:315-325.
25. Fujisawa T, Ikegami H, Ogihara T, Inoue K, Kawabata Y. Effect of two alpha-glucosidase inhibitors, voglibose and acarbose, on postprandial hyperglycemia correlates with subjective abdominal symptoms. Metabol. 2005; 54(3):387-390.
26. Omojate Godstime C, Enwa Felix O, Jewo Augustina O, Eze Christopher O. Mechanisms of Antimicrobial Actions of Phytochemicals against Enteric Pathogens – A Review. J Pharm Chem Biol Sci. August 2014; 2(2):77-85.
27. Tan L, Zhou Y, Huang Y, Wang X, Hao JW. Antimicrobial activity of globulol isolated from the fruits of *Eucalyptus globulusf* Labill. Nat. Prod. Res. 2008; 22: 569-575.
28. Hussein HM, Hameed IH, Ubaid JM. Analysis of the Secondary Metabolite Products of *Ammi majus* and Evaluation Anti-Insect Activity. International Journal of Pharmacognosy and Phytochemical Research. 2016; 8(8):1403-1411.