International Journal of Fauna and Biological Studies
Available online at www.faunajournal.com

I J F B S International Journal of Fauna And Biological Studies

**ISSN 2347-2677**
IJFBS 2019; 6(2): 42-51
Received: 26-01-2019
Accepted: 27-02-2019

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# Phytochemical and GC-MS analysis of *Ziziphus glabrata* Heyne ex Roth (Rhamnaceae)

**K Gokul and V Priya**

## Abstract
The aim of our study was to evaluate the phytochemical and GC-MS analysis of leaf extract of *Ziziphus glabrata.* In this present study, phytochemical analysis was carried in hexane and methanolic extracts of *Z. glabrata* (leaves) was studied. Results of hexane extract showed the presence of alkaloids, steroids and sterols, flavonoids, phenols, cardiac glycosides, fatty acids, glycosides and carbohydrates followed by the methanolic extract showed the presence of alkaloids, steroids and sterols, flavonoids, tannins, cardiac glycosides, fatty acids, terpenoids, phenols and carbohydrates. In this study, GC-MS analysis revealed the presence of 55 bioactive compounds were identified in the methanolic extract.

**Keywords:** *Zizizphus glabrata* leaves, preliminary phytochemical screening and GC-MS analysis

## Introduction
The plants that possess therapeutic properties are generally designated as Medicinal plants (Muhammad Ashikur Rahman *et al.*, 2009) [15]. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceuticals intermediates and chemical entities for synthetic drugs (Ncube *et al.*, 2008) [16]. The medicinal plants have very complex chemical constituents called secondary metabolites, which make them very important in the field of therapeutics (Karthikeyan *et al.*, 2009; Lozoya *et al.*, 1989) [9, 12]. Phytochemicals are the non-nutritive secondary metabolites that have defensive or disease preventive properties (Tan *et al.*, 2010) [24]. Plants synthesize these chemicals to protect themselves. Plants contain a variety of phyto pharmaceuticals, which are found to possess important applications in the field of agriculture, human and veterinary medicine.

*Ziziphus glabrata* belonging to the family Rhamnaceae. It is distributed in Peninsular India, North East India to Bhutan and commonly distributed on stream banks and foothills to 1400 m. It is a tree up to 8 m high, unarmed; branchlets glabrous. Leaves 1.8-10.3 × 1.3-5.2 cm, alternate, lanceolate or ovate-oblong, apex acute, base rounded, crenulate, glabrous, coriaceous, glossy, dark green, basally 3-nerved; petioles 3-9 mm long; stipules filiform, deciduous. Inflorescence axillary fascicles; peduncles 2-3 mm long. Flowers 5-6 mm across, yellowish green, slightly puberulous; pedicles 4-5 mm long. Calyx lobes 2-3 mm long, glabrous inside. Petals obtriangular with convolute margins, 1-2 mm long, acute or rounded at apex. Stamens about 3 mm long; filaments flattened. Disc faintly 10-lobed, glabrous, fleshy. Ovary 2-celled, glabrous; styles 2, united to the middle, curved. Fruits globose, 10-11 mm in diameter, 1-2-celled with a sweet gelatinous pulp. Seeds soft, brownish.

Fruits are well-known for possessing emollient and pectoral properties. Matured fruits are sour but, the dried ones are rather sweet. Pulp of the fruits of the cultivated varieties are sweet, aromatic, mealy and white. People eat ripe fruits. The fruits are also dried in sun, preserved and consumed in off-season. Ripe fruits are also eaten by baking with millet or rice. Decoction of the leaves is applied to purify blood; it is also used in venereal diseases (Bhandari and Bhansali 2000) [3]. It is used to treat inflammation, to relieve pain, convulsions and viral infections (Kirtikar *et al.*, 1975) [10].

As per the literatures still now no work has been reported on this plant regarding the phytochemicals and GC-MS analysis. So, with this knowledge, the aim of our study was to determine the preliminary phytochemical screening and profiling of bioactive compounds using GC-MS method in the leaves of *Ziziphus glabrata.*


Habit


Fruiting


Flowering

**Plate 1:** Morphology of *Ziziphus glabrata* Heyne ex Roth

## Materials and Methods

### Collection of the plant material

The leaves of *Ziziphus glabrata* Heyne ex Roth was collected without any diseases from Kurunthamalai hills, Mettupalayam, Coimbatore, Tamil Nadu. The collected plant was identified and authenticated by the Botanical Survey of India, Southern regional, Coimbatore.

### Preparation of the plant powder and extract

The leaves of *Ziziphus glabrata* was properly washed with water and shade dried at room temperature for 1 month. The dried leaves are grounded into a fine powder with the help of the mechanical device and stored in the air tight container. 30 grams of the leaf powder was subjected to extraction with 250 ml of different solvents (Hexane and Methanol) by Soxhlet apparatus. The leaf extract obtained is stored in two different conical flasks for further analysis.

### Preliminary phytochemical screening

The hexane and methanolic extracts are subjected to preliminary phytochemical tests to determine the group of primary and secondary metabolites like alkaloids, steroids and sterols, flavonoids, triterpenoids, tannins, phenols, cardiac glycosides, saponins, fatty acids, glycosides, terpenoids, proteins, carbohydrates and quinones by the standard protocol described by Harborne, 1973 [6].

### GC-MS analysis

Gas chromatography- Mass spectrometry analysis of the methanolic extract was performed using a GC-MS (Model; QP 2010 ultra series, Shimadu, Tokyo, Japan) equipped with thermal desorption system TD 20. Injection mode: Spilt, Flow Control Mode: Linear Velocity, Pressure: 81.9 kPa, Linear velocity: 40.5 cm/sec, Purge Flow: 3.0 mL/min, Split Ratio: 50.0. For GC-MS detection [GC-2010], Helium gas (99.99%) was used as a carrier gas at a constant flow rate-total flow: 64.7mL/min. and column flow: 1.21mL/min. injector and mass transfer line temperature were set at 200 and 240℃ respectively. The oven temperature was programmed (Column Oven Temperature: 80.0℃ and Injection Temperature: 260.00℃). Total running time of GC-MS is 53 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total area, software adopted to handle mas spectra and chromatograms was a Turbo mass. The relative percentage of each extract constituents was expressed as percentage with peak area.

### Identification of the components

Using computer searches on a NIST Version – Year 2011 were used as MS data library and the mass spectrum of the unknown compound as compared with the spectrum of known compound. The name, molecular structure and molecular weight of the compounds present in the leaf sample were identified.

## Results and Discussion

### Preliminary phytochemical screening

Qualitative phytochemical analysis of hexane and methanolic extracts of *Ziziphus glabrata* leaf revealed the presence and absence of primary and secondary metabolites shown in table 1. The results showed the presence of alkaloids, steroids and sterols, flavonoids, tannins, phenols, cardiac glycosides, fatty acids, terpenoids, proteins, carbohydrates and the absence of triterpenoids, saponins, glycosides and quinones.

**Table 1:** Preliminary phytochemical analysis in *Ziziphus glabrata*

| S. No. | Tests | Hexane extract | Methanolic extract |
|---|---|---|---|
| 1. | Alkaloids | + | + |
| 2. | Steroids and sterols | + | + |
| 3. | Flavonoids | + | + |
| 4. | Triterpenoids | - | - |
| 5. | Tannins | - | + |
| 6. | Phenols | + | - |
| 7. | Cardiac glycosides | + | + |
| 8. | Saponins | - | - |
| 9. | Fatty acids | + | + |
| 10. | Glycosides | - | - |
| 11. | Terpenoids | - | + |
| 12. | Proteins | + | + |

| | | | |
|---|---|---|---|
| 13. | Carbohydrates | + | + |
| 14. | Quinones | - | - |

'+' - presence of the compound
'-' - Absence of the compound

**GC-MS analysis**

The components present in the methanol extract of leaf of *Ziziphus glabrata* were identified through the GC MS method (Fig.1). Fifty-five compounds were present in the leaf of *Z. glabrata* which are belonging to various groups. The active principle with their retention time (RT), compound name, molecular formula, molecular weight, peak area (%) and their molecular structure is given in Table 2. The medicinal uses of the compounds present in the plant is represented in Table 3.

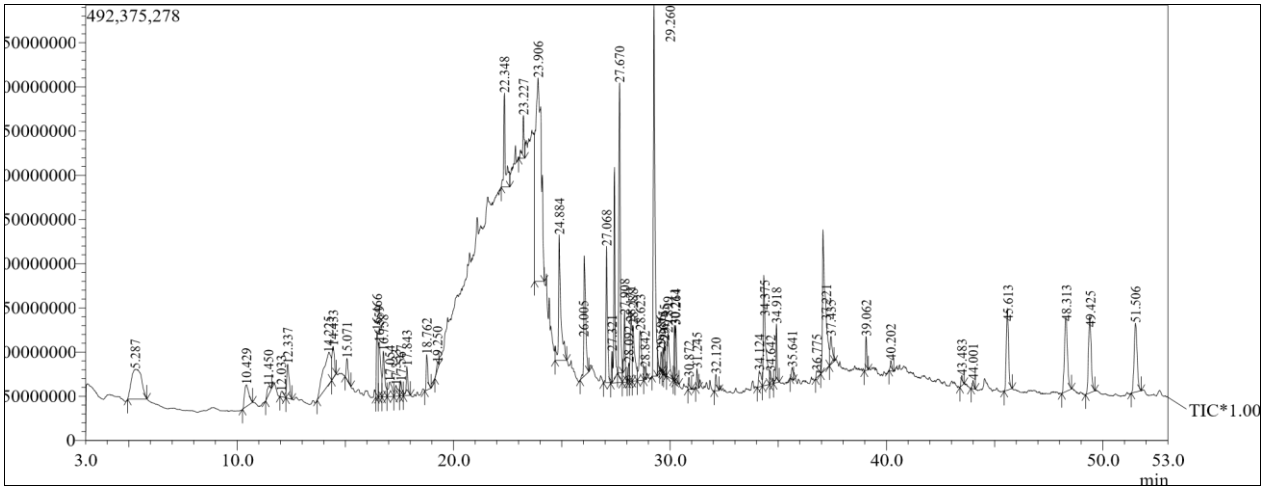
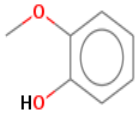
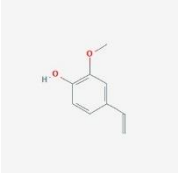
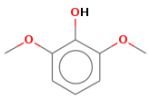
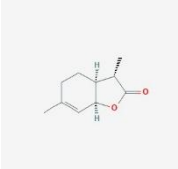
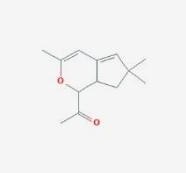
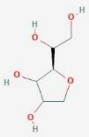
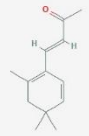
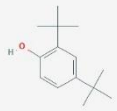
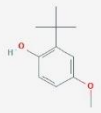
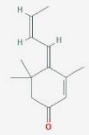
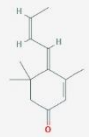
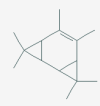
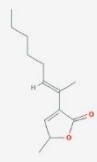



**Fig 1:** Graphical representation of GC MS analysis of *Ziziphus glabrata*

**Table 2:** Phytochemicals obtained from the methanolic extract of *Ziziphus glabrata*

| S. No | RT | Compound name | Formula | Molecular weight | Peak area % | Structure |
|---|---|---|---|---|---|---|
| 1 | 5.287 | Phenol,2-methoxy | $C_7H_8O_2$ | 124.139 g/mol | 4.12 |  |
| 2 | 10.429 | 2-Methoxy-4-vinylphenol | $C_9H_{10}O_2$ | 150.177 g/mol | 1.25 |  |
| 3 | 11.450 | Phenol, 2,6-dimethoxy- | $C_8H_{10}O_3$ | 154.165 g/mol | 0.35 |  |
| 4 | 12.033 | 7,7a-Dimethyl-3a,4,5,7a-tetrahydro-3H-benzofuran-2-one | $C_{10}H_{14}O_2$ | 166.22 g/mol | 0.28 |  |
| 5 | 12.337 | 1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl)-ethanone | $C_{13}H_{18}O_2$ | 206.285 g/mol | 1.12 |  |

| | | | | | | |
|---|---|---|---|---|---|---|
| 6 | 14.225 | d-Mannitol, 1,4-anhydro- | $C_6H_{12}O_5$ | 164.157 g/mol | 0.44 |  |
| 7 | 14.433 | 4-(2,4,4-Trimethyl-cyclohexa-1,5-dienyl)-but-3-en-2-one | $C_{13}H_{18}O$ | 190.286 g/mol | 0.98 |  |
| 8 | 15.071 | Phenol, 2,4-bis(1,1-dimethylethyl)- | $C_{14}H_{22}O$ | 206.329 g/mol | 0.53 |  |
| 9 | 16.466 | 3-tert-Butyl-4-hydroxyanisole | $C_{11}H_{16}O_2$ | 180.247 g/mol | 1.51 |  |
| 10 | 16.758 | Megastigmatrienone | $C_{13}H_{18}O$ | 190.286 g/mol | 1.36 |  |
| 11 | 17.054 | Octahydro-1-benzofuran | $C_8H_{14}O$ | 126.199 g/mol | 0.73 |  |
| 12 | 17.333 | 1,2,2,3-tetramethyl-3-cyclopenten-1-ol | $C_9H_{16}O$ | 140.226 g/mol | 0.46 |  |
| 13 | 17.567 | Megastigmatrienone | $C_{13}H_{18}O$ | 190.286 g/mol | 0.34 |  |
| 14 | 17.843 | Syn-Tricyclo [5.1.0.0(2,4)] oct-5-ene, 3,3,5,6,8,8-hexamethyl | $C_{14}H_{22}$ | 190.33 g/mol | 0.66 |  |
| 15 | 18.762 | 3-(1-Methylhept-1-enyl)-5-methyl-2,5-dihydrofuran-2-one | $C_{13}H_{20}O_2$ | 208.301 g/mol | 0.81 |  |

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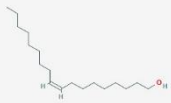
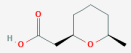
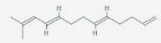
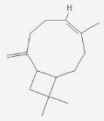
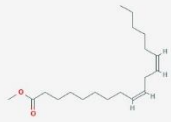
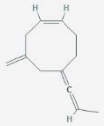
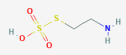
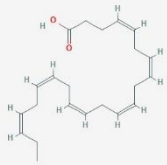
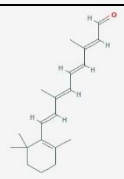
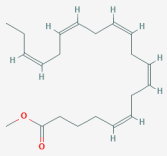
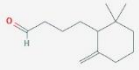
|---|---|---|---|---|---|---|
| 16 | 19.250 | Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate | $C_{16}H_{28}O_3$ | 268.397 g/mol | 0.20 |  |
| 17 | 22.348 | Oleyl Alcohol | $C_{18}H_{36}O$ | 268.485 g/mol | 2.26 |  |
| 18 | 23.227 | 2,6,10-trimethyl,14-ethylene-14-pentadecne | $C_{20}H_{38}$ | 278.524 g/mol | 0.93 |  |
| 19 | 23.906 | 2H-Pyran-2-acetic acid, tetrahydro-6-methyl-, cis | $C_8H_{14}O_3$ | 158.197 g/mol | 15.57 |  |
| 20 | 24.884 | Hexadecanoic acid | $C_{16}H_{32}O_2$ | 256.43 g/mol | 4.02 |  |
| 21 | 26.005 | 1,5,9,11-Tridecatetraene, 12-methyl-, (E, E) | $C_{14}H_{22}$ | 190.33 g/mol | 2.14 |  |
| 22 | 27.068 | Bicyclo [7.2.0] undec-4-ene, 4,11,11-trimethyl-8-methylene-, [1r-(1r*,4e,9s*)] | $C_{15}H_{24}$ | 204.357 g/mol | 2.79 |  |
| 23 | 27.321 | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester | $C_{19}H_{34}O_2$ | 294.479 g/mol | 4.70 |  |
| 24 | 27.670 | Oxirane, hexadecyl | $C_{18}H_{36}O$ | 268.485 g/mol | 5.73 |  |
| 25 | 27.908 | Octadecanoic acid, methyl ester | $C_{19}H_{38}O_2$ | 298.511 g/mol | 1.29 |  |

| | | | | | | |
|---|---|---|---|---|---|---|
| 26 | 28.092 | Adamantane, cyclopropylidene | $C_{13}H_{18}$ | 174.287 g/mol | 0.32 |  |
| 27 | 28.171 | Octadec-9-enoic acid | $C_{18}H_{34}O_2$ | 282.468 g/mol | 1.48 |  |
| 28 | 28.288 | Cyclooctene, 4-methylene-6-(1-propenylidene)- | $C_{12}H_{16}$ | 160.26 g/mol | 1.77 |  |
| 29 | 28.623 | 2-aminoethanethiol hydrogen sulfate (ester) | $C_2H_7NO_3S_2$ | 157.202 g/mol | 1.88 |  |
| 30 | 28.842 | Doconexent | $C_{22}H_{32}O_2$ | 328.496 g/mol | 0.26 |  |
| 31 | 29.260 | Retinal | $C_{20}H_{28}O$ | 284.443 g/mol | 8.50 |  |
| 32 | 29.675 | cis-5,8,11,14,17-Eicosapentaenoic acid, methyl ester | $C_{21}H_{32}O_2$ | 316.485 g/mol | 0.37 |  |
| 33 | 29.745 | 2-Undecenal | $C_{11}H_{20}O$ | 168.28 g/mol | 0.48 |  |
| 34 | 29.899 | 4-(2,2-Dimethyl-6-methylenecyclohexyl) butanal | $C_{13}H_{22}O$ | 194.318 g/mol | 1.27 |  |
| 35 | 30.211 | Cyclopentane-3'-spirotricyclo [3.1.0.0(2,4)]hexane-6'-spirocyclopentane | $C_{14}H_{20}$ | 188.314 g/mol | 0.67 |  |

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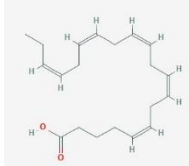
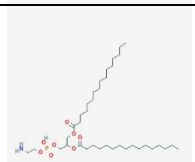
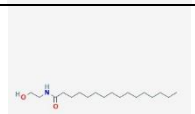
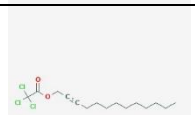
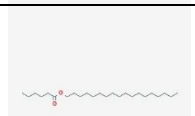
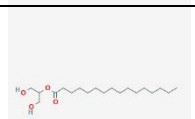
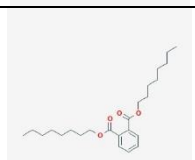
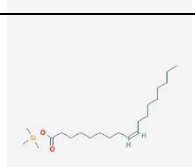
|---|---|---|---|---|---|---|
| 36 | 30.264 | Icosapent | $C_{20}H_{30}O_2$ | 302.458 g/mol | 0.91 |  |
| 37 | 30.872 | Hexadecanoic acid, 1-[[[(2-aminoethoxy) hydroxyphosphinyl] oxy] methyl]-1,2-ethanediyl ester | $C_{37}H_{74}NO_8P$ | 691.972 g/mol | 0.19 |  |
| 38 | 31.245 | Palmidrol | $C_{18}H_{37}NO_2$ | 299.499 g/mol | 0.32 |  |
| 39 | 32.120 | Trichloroacetic acid, tridec-2-ynyl ester | $C_{15}H_{23}Cl_3O_2$ | 341.697 g/mol | 0.35 |  |
| 40 | 34.124 | Hexanoic acid, octadecyl ester | $C_{24}H_{48}O_2$ | 368.646 g/mol | 0.54 |  |
| 41 | 34.375 | Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester | $C_{19}H_{38}O_4$ | 330.509 g/mol | 2.90 |  |
| 42 | 34.642 | Pentadecanal- | $C_{15}H_{30}O$ | 226.404 g/mol | 0.35 |  |
| 43 | 34.918 | Di-n-octyl phthalate | $C_{24}H_{38}O_4$ | 390.564 g/mol | 0.90 |  |
| 44 | 35.641 | 1-Heptatriacotano | $C_{37}H_{76}O$ | 537.014 g/mol | 0.29 |  |
| 45 | 36.775 | Oelsaeure, trimethylsilylester | $C_{21}H_{42}O_2Si$ | 354.65 g/mol | 0.26 |  |

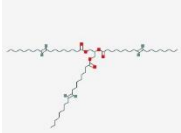
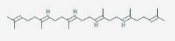
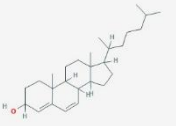
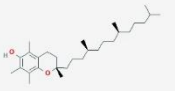
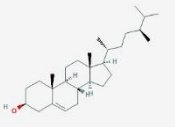
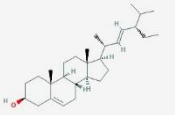
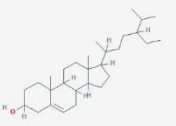
| | | | | | | |
|---|---|---|---|---|---|---|
| 46 | 37.221 | 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E, E, E)- | $C_{57}H_{104}O_6$ | 885.453 g/mol | 3.60 |  |
| 47 | 37.435 | Octadecanoic acid, 2,3-dihydroxypropyl ester | $C_{21}H_{42}O_4$ | 358.563 g/mol | 0.85 |  |
| 48 | 39.062 | Squalene | $C_{30}H_{50}$ | 410.73 g/mol | 0.51 |  |
| 49 | 40.202 | 1,54-Dibromotetrapentacontane | $C_{54}H_{108}Br_2$ | 917.266 g/mol | 0.22 |  |
| 50 | 43.483 | Gamma.-Tocopherol | $C_{28}H_{48}O_2$ | 416.69 g/mol | 0.22 |  |
| 51 | 44.001 | Cholesta-4,6-dien-3-ol, (3. beta.) | $C_{27}H_{44}O$ | 384.648 g/mol | 0.19 |  |
| 52 | 45.613 | dl-. alpha. -Tocopherol | $C_{29}H_{50}O_2$ | 430.717 g/mol | 2.40 |  |
| 53 | 48.313 | Ergost-5-en-3-ol, (3. beta.,24r) | $C_{28}H_{48}O$ | 400.691 g/mol | 2.98 |  |
| 54 | 49.425 | Stigmasterol | $C_{29}H_{48}O$ | 412.702 g/mol | 2.73 |  |
| 55 | 51.506 | Stigmast-5-en-3-ol, (3. Beta.) | $C_{29}H_{50}O$ | 414.718 g/mol | 2.78 |  |

**Table 3:** Medicinal properties of the phytochemicals obtained from the GC-MS analysis of methanolic extract of *Ziziphus glabrata*

| S. No | Compound name | Medicinal properties |
|---|---|---|
| 1 | Phenol,2-methoxy | Antimicrobial (Saravanan *et al.*, 2014) [19]. |
| 2 | 2-Methoxy-4-vinylphenol | Antimicrobial, Antioxidant, Anti-inflammatory, Analgesic (Lee Weng Foo *et al.*, 2015) [11]. |
| 3 | Phenol, 2,6-dimethoxy- | Antiaggregant, anti prostaglandin (Manorenjitha *et al.*, 2013) [13]. |
| 4 | Phenol, 2,4-bis(1,1-dimethylethyl) | Antibacterial activity (Faridha Begum *et al.*, 2016) [4]. |
| 5 | Hexadecanoic acid | Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Anti androgenic, Flavor Hemolytic, 5-Alpha reductase inhibitor (Jananie *et al.*, 2011) [7]. |
| 6 | Oxirane, hexadecyl | Antibacterial, antimicrobial, antioxidant, antipyretic, anti-inflammatory, analgesic (Sunita Arora *et al.*, 2017) [23]. |
| 7 | Octadecanoic acid, methyl ester | Antioxidant (Meechaona, 2007) [14]. |
| 8 | Octadec-9-enoic acid | Anti-inflammatory, Anti-alopecic, Haemolytic and 5-Alpha reductase inhibitor, lubricant, Antitumour, Immunostimulant, Antiandrogenic, Antibacterial, Antifungal, Lipoxygenase inhibitor, Diuretic (Omotoso Abayomi *et al.*, 2014) [17]. |
| 9 | 2-Undecenal | Antimicrobial (Lee Weng Foo *et al.*, 2015) [11]. |
| 10 | Palmidrol | Antioxidants, antiallergic agent, anti-inflammation, skin treatment, anticancer, drugs for disorders of the urinary system, also used for genital or sexual disorders (Saturnino *et al.*, 2017) [20]. |
| 11 | Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester | Antioxidant (Sunita Arora and Ganesh Kumar, 2017) [23]. |
| 12 | Pentadecanal | Antibacterial, antioxidant (Varsha Jadhav *et al.*, 2014) [25]. |
| 13 | Di-n-octyl phthalate | Antifouling, antimicrobial (Arockia Jenecius *et al.*, 2012) [2]. |
| 14 | 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E, E, E) | Anti-spasmodic and immune modulators (Ali Hussein Al-Marzoqi *et al.*, 2016) [1]. |
| 15 | Octadecanoic acid, 2,3-dihydroxypropyl ester | Anticancer, antimicrobial (Sunita Arora and Ganesh Kumar, 2017) [23]. |
| 16 | Squalene | Anticancer, Antimicrobial, Antioxidant Chemo preventive, Pesticide Anti- tumor and Sunscreen (Jananie *et al.*, 2011) [7] |
| 17 | Gamma.-Tocopherol | Anticancer, antioxidant, antitumor, anti-inflammatory, hypocholesterolemia, cardioprotective (Shahina parveen *et al.*, 2016) [21]. |
| 18 | Cholesta-4, 6-dien-3-ol, (3. beta.) | Antimicrobial, Diuretic, Anti-inflammatory, Anti-asthma |
| 19 | dl-.alpha.-Tocopherol | Antioxidant property, Anticancer, Antitumor, Anti-mutagenic, Antidiabetic, Anti-Infertility, Antiparkinsonian, Antialzheimeran, Antiatherosclerosis, Hepatoprotective, Cardioprotective, Immunomodulator, Antistroke, Vasodilator. |
| 20 | Ergost-5-en-3-ol, (3. beta.,24r) | Antimicrobial, anti-inflammatory effects. |
| 21 | Stigmasterol | Antimicrobial, Anticancer, Antiarthritic, Antiasthma, Diuretic, Anti-inflammatory (Sudha *et al.*, 2013) [22]. |
| 22 | Stigmast-5-en-3-ol, (3. Beta.) | Antimicrobial antioxidant anti-inflammatory antiarthritic antiasthma. |

## Conclusion

The present study was to establish the phytochemical and GC-MS analysis of leaves of *Ziziphus glabrata* which contains the primary and secondary metabolites like alkaloids, steroids and sterols, flavonoids, tannins, cardiac glycosides, phenols, terpenoids, fatty acids, proteins and carbohydrates identified through preliminary phytochemical analysis. These primary and secondary metabolites are the important constituents of pharmacological activities. Whereas in GC-MS analysis totally 55 bioactive compounds were identified in the methanol extract. To the best of my knowledge, till now there were no reports on phytochemical and GC-MS study of leaves of *Z. Glabrata.* Therefore, this study will be the basic step for future studies.

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