Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

# Phytochemical Screening, *In-vitro* Anti-oxidant Activity and Gas Chromatography–Mass spectrometry Analysis of Methanolic Extracts of *Smilax perfoliata* and *Breynia retusa*

**Mariyam Begum, Kanjarla Narasimha***

*Chaitanya deemed to be University, Hanamkonda, Warangal, Telangana, India.*

ARTICLE INFO

**Article history:**

Received: 02 October, 2022

Revised: 22 October, 2022

Accepted: 28 October, 2022

Published: 30 November, 2022

**Keywords:**

*Breynia retusa*, GC-MS, Hydroxyl radaical scavenging, *Smilax perfoliata*, *In-vitro* antioxidant activity, Metal chelating activity.

**DOI:**

10.25004/IJPSDR.2022.140618

ABSTRACT

The present studies focus on identification of bioactive compounds from methanolic extraction of *Smilax perfoliata and Breynia retusa* by GC–MS analysis and also evaluated the phytochemical constituents present in *S. perfoliata and B. retusa* methanolic extraction. *In-vitro* antioxidant activity (hydroxyl radaical scavenging activity, determination of reducing power, metal chelating activity, carbon tetrachloride ($CCl_4$) induced lipid peroxidation, inhibitory test on protein oxidative modification, lipid peroxidation assay, catalase assay and reduced glutathione assay) was evaluated by adopting different methods. Phytochemical screening of the methanolic extraction of *S. perfoliata* and *B. retusa* revealed the presence of flavonoids, phenolic compounds, triterpenoids, tannins, saponins, amino acids, proteins, steroids and carbohydrates gas chromatography-mass spectrometry (GC-MS) analysis of the methanol extract of *S. perfoliata* has shown the presence of different bio active compounds. A total of 24 compounds were identified in methanolic extract composition S. *perfoliata* showed bio active compounds like propanimidamide, N-(1-chloro-1-propenyl)-, monohydrochloride, 5-bromo-8-(5-nitrosalicylideneamino) quinoline hydrochloride, nitrogen fluoride (N2F2), (Z)-20 1.426, 2,4,6-cycloheptatrien-1-one, hexahydro-isobenzofuran-1-one, ethene, 1-(2-methoxy-,3,5-dibromophenyl)-2-nitro- was found. And *B. retusa* showed bio active compounds like acetyl bromide, N, N'-ethylenebis (N-nitroacetamide), 2-propanone, 1,1-dichloro-, N,N'-ethylenebis(N-nitroacetamide), 2-propanone, 1,1-dichloro-methane, chlorotrinitro- was found. These compounds are responsible for the different therapeutic and pharmacological properties. Methanolic extraction of *S. perfoliata and B. retusa* possesses antioxidant activity.

## Introduction

Modern-day synthetic and chemical drugs are often explored with hesitate as they exhibit side effects,[1] while traditional herbals are gaining huge interests as they are more natural, environment-friendly and devoid of side effects.[2] Hence, with all the benefits of modern synthetic medicines, people have still preferred plant-based natural medicines over synthetic medicines.[3] Most of the medicinal plants are distinctive in their ability to treat, as well as to cure various human ailments owing to the contribution of various valuable phytoconstituents present in different plant parts.[3,4,5] In India, from ancient time, different parts of medicinal plants (~ 80,000 species) have been used as traditional medicines in different systems of Indian medicines for treatments of various diseases.[6] At present, about 25% of the active constituents have been identified from medicinal plants, which have been used as prescribed medicines.[7] Certain reports have estimated that over 25,000 of actual plant-based formulations are available in the Indian systems of folk and traditional medicine, which are prescribed by about 1.5 million practitioners in preventive, persuasive and healing applications.[7,8]

Various bioactive compounds of medicinal plants exhibit stimulating pharmacological actions like antibacterial,

***Corresponding Author:** Dr. Kanjarla Narasimha

**Address:** Chaitanya deemed to be University, Hanamkonda, Warangal-506001, Telangana, India.

**Email** ✉: mariyambegum331@gmail.com

**Tel.:** +91-8919509603

**Relevant conflicts of interest/financial disclosures:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

antifungal, anticancer, anti-inflammatory and antioxidant properties.[3,4,9] The potential of these bioactive compounds should be analyzed for their candidature in treatments of various ailments.[4,7] Plant-based medicines are often prepared from crude plant extracts comprising of complex mixture of different phytochemicals.[7] These phytochemicals have unique and complex structures, and are used in treating prolonged as well as contagious diseases.[2,7] An enormous pool of bioactive secondary metabolites exists in various plant species, but merely a small proportion of them have been examined and sustained to be significant source of bioactive agents. In the search for new compounds, and also for quality control, development of suitable screening methods is very important.[10] Extractions and characterizations of numerous such bioactive compounds from various medicinal plants have led to the delivery of certain medicines with high-activity profile.[11]

The initial screening of medicinal plants by spectrometric and chromatographic methods provides basic information on chemical and pharmacological activities, which helps to select the biologically active plants.[12] In recent years, fourier-transform infrared (FTIR) and gas chromatography-mass spectrometry (GC–MS) has commonly been employed for detection of functional groups and identification of various bioactive therapeutic compounds that are present in medicinal plants.[13,14] GC–MS is one of the best, fast and accurate techniques to detect various compounds, including alcohols, alkaloids, nitro compounds, long chain hydrocarbons, organic acids, steroids, esters and amino acids,[15] and requires a small volume of plant extracts. Hence, in the present study, GC–MS technique was adopted for detection and identification of phytochemical compounds present in the medicinal plant.

*Smilax* (Family -*Smilacaceae)* is a large genus of climbing shrub distributed in tropical and temperate regions of the world. *Smilax perfoliata* Lour is found in various parts of India and has tuberous rhizomes. It is a robust more or less strongly armed climber. Stem is used as toothbrush to strengthen the gums. Tender shoot is taken in curries and is useful as blood purifier. Roots and stems are used as anticancer, anti-dysenteric and in urinary complaints. *Breynia retusa* is commonly known as common name: cup saucer plant, cupped coral-berry tree is a shrub with spreading branches. The common names of this plant originate from this. *Breynia* is a plant genus in the family *Phyllanthaceae*, first described as a genus in 1776. *B. retusa* is native to Southeast Asia, china, they are found throughout tropical and subtropical regions of the world. It has long been used in Indian folk medicine to treat broad spectrum of diseases. Which are concentrated in parts like bark, leaves, roots and seeds. Plants of this family have been used to treat cancer. So far, various phytochemical reported from in this review importance of this species and scope for taking research with this plant is discussed. Phytochemical studies on *Breynia retusa* disclosed major chemical constituents including this species such as reducing sugars, phenolics (most abundant phenols are gallic acid, ellagic acid, coumaric acid, ferullic acid and vanillic acid), alkaloids, tannins, glycosides, flavones and saponins, proteins. A research report on *B. retusa* reveals for its dysentery, toothache, skin inflammation, hyperglycemia, diarrhoea and as astringent and diuretic, anti-arthritic effect.

Many drugs have failed to enter market owing to their poor pharmacokinetic properties, which incurs huge losses to pharmaceutical companies. Computer-aided tools have emerged as advanced methods for drug discovery, which can be applied to screen the drugs from phytochemicals found in various medicinal plants. Computational prediction models, also known as predictive tools, which play an important part in the selection of methodologies directing pharmaceutical and technological research, and have also been used in *in-silico* prediction of pharmacological, pharmacokinetic and toxicological performance.[16] Currently, molecular docking is an effective and inexpensive method for designing and testing the drugs. This technique provides information about drug receptor interactions that are useful to predict the binding orientation of drug candidates to their target proteins.[17] Besides, this approach helps in systemic study by introducing a molecule on the binding spot of the object macromolecule in a non-covalent fashion, leading to an accurate binding at the active sites of each ligand.[18]

Therefore, the present study focuses on identification of bioactive compounds from methanolic extraction of *S. perfoliata* and *B.* retusa by GC–MS analysis.

## Materials And Methods

### Plant Material

*S. perfoliata roots* and *Breynia retusa* bark were authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati and voucher specimen was (Pt 0815 & Pt 0846) preserved in the herbarium.

### Extraction of Plant Material

Five kilogram of the fresh plant material was shade dried at temperatures 25–35°C for 7 days. The dried plant material was powdered in a grinder. The dried plant powder was subjected to soxhlet extraction using n-hexane, ethyl acetate and methanol to get the respective extracts. Then each of the extracts was filtered using cotton plugs followed by whatman no. 1 filter paper. The filtrates were then concentrated, dried under reduced pressure in the rotary evaporator and lyophilized to get in powder form.

## Percentage Yield

The percentage of yield was calculated using the following formula: -

$$\text{Yield (g/100 g)} = (W_1 \times 100)/W_2$$

Where,

$W_1$ = weight of the crude extract residue obtained after solvent removal

$W_2$ = weight of plant powder packed in the soxhlet

## Preliminary Phytochemical Analysis

All the extract/fractions of roots of S. *perfoliata* and bark *of B. retusa* were analyzed for their primary and secondary metabolites to confirm the presence of various primary metabolites, such as carbohydrates, amino acids, proteins, and lipids, and secondary metabolites, such as alkaloids, tannins, phenols, flavonoids, saponins, steroids, glycosides, and resins, according to standard methods.

## Gas Chromatography-mass Spectrometry (GC-MS) Analysis

GC-MS analysis was carried out in a combined 7890A gas chromatograph system (GCMSQP2010, SHIMADZU) and mass spectrophotometer, fitted with a HP-5 MS fused silica column (5% phenyl methyl siloxane 30.0 m ×250 µm, film thickness 0.25 µm), interfaced with 5675C Inert MSD with triple-axis detector. Helium gas was used as carrier gas and was adjusted to column velocity flow of 1.0 mL/min. Other GC-MS conditions are ion-source temperature, 250°C; interface temperature, 300°C; pressure, 16.2 psi; out time, 1.8 mm; and 1-µL injector in split mode with split ratio 1:50 with injection temperature of 300°C. The column temperature started at 36°C for 5 minutes and changed to 150 V at the rate of 4°C/min. The temperature was raised to 250°C at the rate of 20°C/min and held for 5 minutes. The total elution was 37 minutes. The relative percent amount of each component was calculated by comparing its average peak area to total areas. MS solution software provided by supplier was used to control the system and to acquire the data.

## Identification of Compounds

Identification of components was achieved based on their retention indices and interpretation of mass spectrum was conducted using the database of National Institute of Standards and Technology (NSIT). The database consists of more than 62,000 patterns of known compounds. The spectra of the unknown components of S. *perfoliata* and *B. retusa* fraction obtained were compared with the standard mass spectra of known components stored in NIST library (NISTII).

## *In-vitro* Antioxidant Studies

### *Hydroxyl Radaical Scavenging Activity*

The hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extract for hydroxyl radicals generated from the $Fe^{3+}$/ascorbate/EDTA/$H_2O_2$ system. The hydroxyl radicals attack deoxyribose, which eventually results in TBARS formation. The reaction mixture contained deoxyribose (2.8 mM), $FeCl_3$ (0.1 mM), $H_2O_2$ (1 mm), ascorbate (0.1 mM), $KH_2PO_4$-KOH buffer (20 mM, pH 7.4) and various concentrations (MESP and MEBR 100, 200, and 300 µg/mL and standard mannitol 100 µg/mL) of the drug in a final volume of 1-mL. The reaction mixture was incubated for 1-hour at 37°C. Deoxyribose degradation was measured at 532 nm.[19]

## Determination of Reducing Power

The reducing power of MESP and MEBR was determined according to the following method. Various concentrations (125, 250, 175 and 500 µg/mL) of extract of MESP and MEBR in 1-mL of distilled water was mixed with phosphate buffer (2.5 mL, 0.2M, pH 6.6) and potassium ferricyanide ($K_3Fe(CN)_6$) (2.5 mL, 1%). The mixture was incubated at 50°C for 20 minutes. A portion (2.5 mL) of trichloroacetic acid (15%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increased reducing power.[20]

## Metal Chelating Activity

Metal chelation property for ferric ion ($Fe^{3+}$) was estimated by using thiocynate method. Here different ratio of the extract (1:0.25 to 1:10 ratio) was mixed with a fixed concentration of ferric chloride (10 µg). The mixture was incubated for 30 minutes. At the end of the incubation, 1-mL of potassium thiocyanate (25%) was added and absorbance of ferric-thiocyanate complex (reddish brown complex) was measured at 460 nm. The results were compared with EDTA (1:10). Metal chelation property for ferrous ion ($Fe^{2+}$) was estimated by using 2, 2-bipyridyl method. Here different concentrations of the extract were mixed with a fixed concentration of ferrous sulphate (10 µg). The mixture was incubated for 30 minutes. At the end of the incubation, 2 mL of 2, 2-bipyridyl (1 mM) was added and absorbance of ferrous– bipyridyl complex (pink-colored complex) was measured at 525 nm. The results were compared with EDTA.[21]

## Carbon Tetrachloride ($CCl_4$) Induced Lipid Peroxidation

Rat liver (30%w/v) homogenate in ice–cold 0.15 M potassium chloride was prepared in homogenizer. Aliquots of 0.5 mL of homogenate were taken in different small conical flasks. These flasks were incubated at 37°C in a constant shaker bath (150 cycles/min) for 45 minutes with 1.5 mL of potassium phosphate buffer (pH 7.4), 2 mL of 0.15M potassium chloride, MESP and MEBR at (25, 50, 100, 200 and 300 µg/mL) and vitamin – E 100 µg/mL in different flasks and finally 10 µL of carbon tetrachloride ($CCl_4$) was added. In case of control, both $CCl_4$ and drugs were not added and in some flasks only drug was excluded.

The reaction was stopped by the addition of 4 mL of 10% (w/v) tri chloro acetic acid and after incubation, the contents were centrifuged at 4000 rpm for 10 minutes and about 2 mL of clear supernatant was transferred to a graduated tube and 2 mL of 0.67% w/v of thiobarbituric acid was added and heated in a boiling water bath for 15 minutes. The tubes were cooled, bringing the mixture to pH 12–12.5 with potassium hydroxide, stabilized the colour developed, and the absorbency was measured at 543 nm.[22]

### Inhibitory Test on Protein Oxidative Modification

Albumin oxidative modification by copper was performed by the following method. The test sample (MESP and MEBR 100–1000 µg/mL) and vitamin- E (100–1000 µg/mL), was added to the reaction mixture containing albumin (10 µg/mL) and 100 µM $CuCl_2$ in 50 mM tris- HCL buffer (pH 7.4) in a total volume 0.3 mL. The mixture was incubated at 37°C for 2 hour. Next 1.6 mL of 0.125 M phosphate buffer (pH 8.0) is containing 12.5 mM EDTA and 10.0 M urea, and 0.1 mL of 50 mm phosphate buffer (pH 7.0) containing 10 mM DTNB were added to the reaction mixture. This solution was allowed to stand at room temperature for 5 minutes. The absorbency was read at 412 nm as cysteine-SH residue.[23]

## RESULTS

Percentage yields of n-hexane, ethyl acetate and methanolic extractions of *S. perfoliata roots and B. retusa* bark results were showed in Table 1.

In the present study, the investigation of n-hexane, ethyl acetate and methanolic extraction of S. *perfoliata* and *B. retusa* revealed the presence of various presences of various phytoconstituents like flavonoids, phenolic compounds, triterpenoids, tannins, saponins, amino acids, proteins, and carbohydrates results were showed in Table 2 and 3. Based on the Phytochemical analysis and percentage yield of extractions selected methanolic extraction was selected both plants for further GC-MS analysis.

GC-MS investigation of methanolic extraction of S. *perfoliata* and *B. retusa* showed different phytochemicals. The chromatograms displayed in Fig. 1 and 2 whereas the chemical constituents with their retention time (RT), atomic equation, molecular weight (MW) and area (%) within the MESP and MEBR are displayed in Table 4 and 5. The following bioactive compounds were present in the GC-MS analysis carried on methanolic fraction of S. *perfoliata* showed bio active compounds like propanimidamide, N-(1-chloro-1-propenyl)-, monohydrochloride, 5-bromo-8-(5-nitrosalicylideneamino) quinoline hydrochloride, nitrogen fluoride (N2F2), (Z)-20 1.426, 2,4,6-cycloheptatrien-1-one, hexahydro-isobenzofuran-1-one, ethene, 1-(2-methoxy-,3,5-dibromophenyl)-2-nitro- was found, and *Breynia retusa* showed bio active compounds like acetyl bromide, N, N'-ethylenebis (N-nitroacetamide), 2-propanone, 1,1-dichloro-, N,N'-ethylenebis (N-nitroacetamide), 2-Propanone, 1,1-dichloro-methane, chlorotrinitro- was found.

**Table 1:** Percentage yield

| Extract | n-hexane extract | Ethyl acetate extract | Methanol extract |
|---|---|---|---|
| *S. perfoliata roots* | 0.17 | 1.12 | 2.04 |
| *B. retusa* bark | 1.14 | 2.42 | 4.18 |

**Table 2:** Results of Phytochemical screening of n-Hexane, Ethyl acetate and Methanolic extraction of *S. perfoliata*

| *S. No* | *Name of the Phytochemical* | *n-Hexane,* | *Ethyl acetate* | *Methanol* |
|---|---|---|---|---|
| 1 | Carbohydrates | + | + | + |
| 2 | Amino acids | + | + | + |
| 3 | Proteins | - | - | + |
| 4 | Alkaloids | + | + | + |
| 5 | Cardiac glycosides | - | - | + |
| 6 | Triterpenoids | + | + | + |
| 7 | Saponins | + | + | + |
| 8 | Flavonoids | - | - | + |
| 9 | Phenolic compounds | + | + | + |
| 10 | Tannins | + | - | + |
| 11 | Steroids | + | + | + |
| 12 | Gums | - | - | - |

Where, + means positive and - means negative.

**Table 3:** Results of Phytochemical screening of n-Hexane, Ethyl acetate and Methanolic extraction of *B. retusa*

| *S. No* | *Name of the Phytochemical* | *n-Hexane,* | *Ethyl acetate* | *Methanol* |
|---|---|---|---|---|
| | Carbohydrates | + | + | + |
| | Amino acids | + | + | + |
| | Proteins | - | - | + |
| | Alkaloids | + | + | + |
| | Cardiac glycosides | - | - | + |
| | Triterpenoids | - | + | + |
| | Saponins | + | - | + |
| | Flavonoids | - | - | + |
| | Phenolic compounds | + | + | + |
| | Tannins | + | - | + |
| | Steroids | + | + | + |
| | Gums | - | - | - |

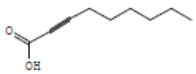
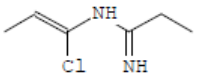
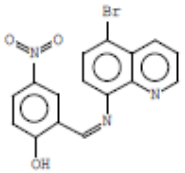
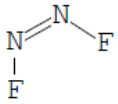
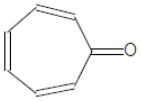
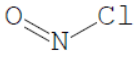
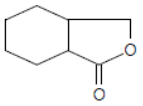
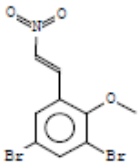
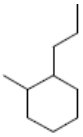
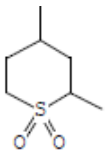
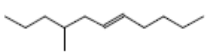
Where, + means positive and - means negative.

### *In-vitro* Anti-oxidant Study

#### *Hydroxyl Radical Scavenging Activity of MESP and MEBR*

When compared to the control, the MESP and MEBR considerably ($p < 0.001$) scavenged the hydroxyl radicals produced by the EDTA/$H_2O_2$ system (at all tested doses of 100, 200, and 300 g). The amount of OH radicals that MEPM was able to scavenge increased in a dose-dependent way. Comparable results were obtained using nannitol 100 ug (*$p < 0.001$). The results are displayed in Table 6.

**Table 4:** Bioactive compounds found in Methanolic extract of *Smilax Perfoliata* (MESP)

| *S. No* | *R. Time* | *Area%* | *Compound name* | *Molecular Formula* | *M.W g/ mol* | *Structure of Compound* |
|---|---|---|---|---|---|---|
| 1 | 0.035 | 11.35 | 2-nonynoic acid | $C_9H_{14}O_2$ | 154 |  |
| 2 | 0.150 | 3.83 | Propanimidamide, N-(1-chloro-1-propenyl)-, monohydrochloride | $C_6H_{12}C_{l2}N_2$ | 182 |  |
| 3 | 0.230 | 6.02 | 5-Bromo-8-(5-nitrosalicylideneamino) quinoline hydrochloride | $C_{16}H_{10}BrN_3O_3$ | 371 |  |
| 4 | 1.204 | 0.50 | Methylene Chloride | $CH_2Cl_2$ | 84 |  |
| 5 | 1.346 | 0.54 | Nitrogen fluoride (N2F2), (Z)- 20 1.426 | $F_2N_2$ | 66 |  |
| 6 | 1.625 | 0.20 | 2,4,6-Cycloheptatrien-1-one | $C_7H_6O$ | 106 |  |
| 7 | 2.155 | 0.24 | Nitrosyl chloride | ClNO | 65 |  |
| 8 | 2.260 | 0.37 | Hexahydro-isobenzofuran-1-one | $C_8H_{12}O_2$ | 140 |  |
| 9 | 2.478 | 44.47 | Ethene, 1-(2-methoxy-,3,5-dibromophenyl)-2-nitro- | $C_9H_7Br_2NO_3$ | 335 |  |
| 10 | 2.530 | 0.28 | Cyclohexane, 1-methyl-2-propyl- | $C_{10}H_{20}$ | 140 |  |
| 11 | 2.604 | 0.15 | trans-2,4-Dimethylthiane, S,S-dioxide | $C_7H_{14}O_2S$ | 162 |  |
| 12 | 2.661 | 0.26 | 5-Undecene, 8-methyl-, (E)- | $C_{12}H_{24}$ | 168 |  |

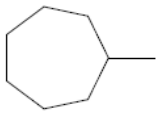
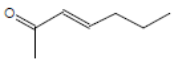
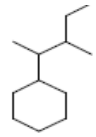
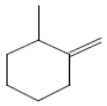
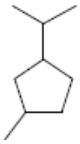
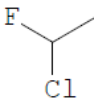
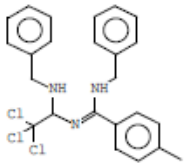
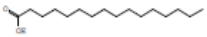
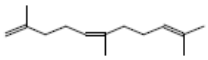
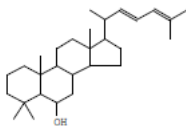
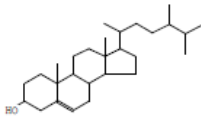
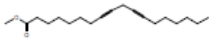
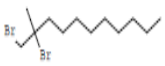
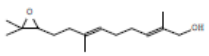
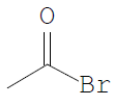
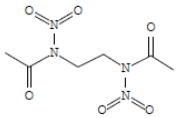
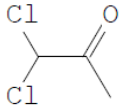
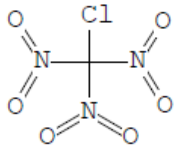
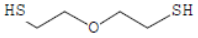
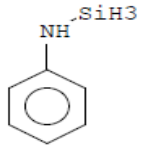
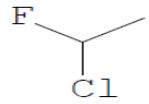
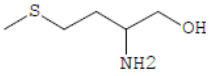
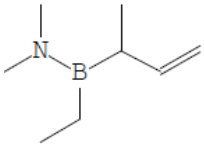
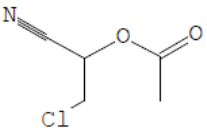
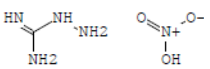
| | | | | | | |
|---|---|---|---|---|---|---|
| 13 | 2.730 | `1.05 | Cycloheptane, methyl- | $C_8H_{16}$ | 112 |  |
| 14 | 2.809 | 0.90 | 3-Hepten-2-one, (E)- | $C_7H_{12}O$ | 112 |  |
| 15 | 3.002 | 0.62 | Cyclohexane, (1,2-dimethylbutyl)- | $C_{12}H_{24}$ | 168 |  |
| 16 | 3.051 | 0.33 | 1-Methyl-2-methylenecyclohexane | $C_8H_{14}$ | 110 |  |
| 17 | 3.140 | 1.00 | Cyclopentane, 1-methyl-3-(1-methylethyl)- | $C_9H_{18}$ | 126 |  |
| 18 | 3.510 | 0.13 | Ethane, 1-chloro-1-fluoro- | $C_2H_4ClF$ | 82 |  |
| 19 | 3.544 | 0.85 | N-Benzyl-N'-(1-benzylamino-2,2,2-trichloroethyl)-p-tolylcarboxamidine | $C_{24}H_{24}Cl_3N_3$ | 459 |  |
| 20 | 21.13 | 0.25 | n-Hexadecanoic acid | $C_{16}H_{32}O_2$ | 256 |  |
| 21 | 28.712 | 0.61 | 1,5,9-Undecatriene, 2,6,10-trimethyl-, (Z)- | $C_{14}H_{24}$ | 192 |  |
| 22 | 31.636 | 0.65 | Cholesta-22,24-dien-5-ol, 4,4-dimethyl- | $C_{29}H_{48}O$ | 412 |  |
| 23 | 32.066 | 2.40 | 5-Cholestene-3-ol, 24-methyl- | $C_{28}H_{48}O$ | 400 |  |
| 24 | 32.735 | 0.21 | 8,11-Octadecadiynoic acid, methyl ester | $C_{19}H_{30}O_2$ | 290 |  |
| 25 | 34.116 | 2.61 | Undecane, 1,2-dibromo-2-methyl- | $C_{12}H_{24}Br_2$ | 326 |  |
| 26 | 36.138 | 1.16 | 9-(3,3-Dimethyloxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-ol | $C_{15}H_{26}O_2$ | 238 |  |

**Table 5:** Bioactive compounds found in Methanolic extract of *Breynia retusa* (MEBR).

| *S. No* | *R. Time* | *Area%* | *Compound name* | *Molecular Formula* | *M.W g/mol* | *Structure of Compound* |
|---|---|---|---|---|---|---|
| 1 | 0.075 | 5.75 | Acetyl bromide | $2H_3BrO$ | 122 |  |
| 2 | 0.521 | 0.64 | N,N'-Ethylenebis(N-nitroacetamide) | $C_6H_{10}N_4O_6$ | 234 |  |
| 3 | 0.510 | 1.29 | 2-Propanone, 1,1-dichloro- | $C_3H_4Cl_2O$ | 126 |  |
| 4 | 0.725 | 0.25 | Methane, chlorotrinitro- | $CClN_3O_6$ | 185 |  |
| 5 | 0.835 | 0.45 | 2-Mercaptoethyl ether | $C_4H_{10}OS_2$ | 138 |  |
| 6 | 1.168 | 1.18 | Silanamine, N-phenyl- | $C_6H_9NSi$ | 123 |  |
| 7 | 1.390 | 1.54 | Dodecane, 1-fluoro- | $C_{12}H_{25}F$ | 188 |  |
| 8 | 1.430 | 0.13 | Ethane, 1-chloro-1-fluoro- | $C_2H_4ClF$ | 82 |  |
| 9 | 1.460 | 2.01 | L-Methioninol | $C_5H_{13}NOS$ | 135 |  |
| 10 | 1.654 | 0.67 | Boranamine, 1-ethyl-N,N-dimethyl-1-(1-methyl-2-propenyl)- | $C8H_{18}BN$ | 139 |  |
| 11 | 1.875 | 0.24 | Lactonitrile, 3-chloro-, acetate | $C_5H_6ClNO_2$ | 147 |  |
| 12 | 1.985 | 0.15 | Hydrazinecarboximidamide, nitrate | $CH_7N_5O_3$ | 137 |  |

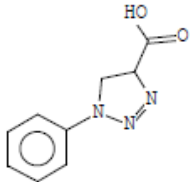
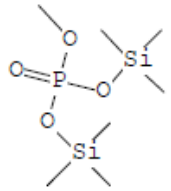
| | | | | | | |
|---|---|---|---|---|---|---|
| 13 | 2.455 | 5.81 | 1H-1,2,3-Triazole-4-carboxylic acid, 4,5-dihydro-1-phenyl- | $C_9H_9N_3O_2$ | 191 |  |
| 14 | 11.666 | 0.24 | Phosphoric acid, bis(trimethylsilyl)monomethyl ester | $C_7H_{21}O_4PSi_2$ | 256 |  |

**Table 6:** Hydroxyl radical scavenging activity MESP, MEBR and Mannitol

| *S. No* | *Concentration (µg/mL)* | *%inhibition of hydroxyl radical* |
|---|---|---|
| 1 | Control | - |
| 2 | MESP (200) | 76.32 ± 4.54 |
| 3 | MESP (400) | 82.32 ± 6.24 |
| 4 | MEBR (200) | 75.92 ± 5.23 |
| 5 | MEBR (400) | 81.63 ± 7.32 |
| 6 | Standard (Mannitol 100µg) | 81.56 ± 5.85 |

### *Determination of Reducing Power*

With higher MESP and MEBR concentrations reducing power increased. Significant (*$p < 0.001$) activity was seen at all tested MESP and MEBR concentrations compared to the control. The outcomes were similar to the benchmark (BHT) (*$p < 0.001$). The results are displayed in Table 7.

### *Effect of MESP and MEBR on $fe^{2+}$ and $fe^{3+}$ Metal Chelation*

At a 1:10 ratio of iron was found in MESP chelated Fe2+ (55.75 ± 3.35%) and Fe3+ (66.24 ± 3.26) and MESP chelated $Fe^{2+}$ (57.96 ± 3.74%) and Fe3+ (67.95 ± 2.16). MESP and MEBR increased dose-dependently, as did the chelating power for mental transition ions ($Fe^{2+}$, $Fe^{3+}$). When compared to the control, MESP and MEBR showed considerable (*$p$ 0.001) chelation at all tested doses. When compared to the control, EDTA showed considerable (*$p < 0.001$) chelation of $Fe^{2+}$ and $Fe^{3+}$ of 78.64 and 85.42%, respectively, under the same circumstances. The results are displayed in Table 8.

### *Lipid Peroxidation Induced By $CCl_4$*

When compared to the control, MESP and MEBR significantly (*$p < 0.001$) reduced the generation of lipid peroxide from $CCl_4$ at all tested dose levels (25, 50, 100, 200, and 300 g). In a dose-dependent way, the percentage inhibitions of peroxide generation rose. The outcomes were comparable to those of the norm. The results are displayed in Table 9.

**Table 7:** Determination of reducing power of MESP, MEBR and BHT

| *S. No* | *Concentration (µg/mL)* | *Absorbance* |
|---|---|---|
| 1 | Control | 0.086 ± 0.00013 |
| 2 | MESP (400) | 1.042 ± 0.00024 |
| 3 | MESP (375) | 0.965 ± 0.00032 |
| 4 | MESP (200) | 0.542 ± 0.00076 |
| 5 | MESP (125) | 0.324 ± 0.00087 |
| 6 | MEBR (400) | 1.032 ± 0.00042 |
| 7 | MEBR (375) | 0.953 ± 0.00064 |
| 8 | MEBR (200) | 0.553 ± 0.00032 |
| 9 | MEBR (125) | 0.342 ± 0..00053 |
| 10 | BHT (400) | 0.634 ± 0.00025 |
| 11 | BHT (375) | 0.512 ± 0.00042 |
| 12 | BHT (200) | 0.395 ± 0.00015 |
| 13 | BHT (125) | 0.496 ± 0.00036 |

### *Inhibitory Test on Protein Oxidative Modification*

At a concentration of 1000 g/mL, the inhibitory ratio of MESP and MEBR on albumin oxidative modification reached a maximum of 78.94 and increased in a concentration-dependent way. It was discovered that MESP $IC_{50}$ was 426.35 ± 4.56 g/mL and MEBR $IC_{50}$ was 430.16 ± 7.62 g/mL. At a concentration of 1000 g/mL, the results were equivalent to the industry standard (mannitol), with a percentage inhibitory ratio of 81.99%. Mannitol's $IC_{50}$ was determined to be 263.35 7.41 g/mL. The results are displayed in Table 10.

ANOVA was used as the statistically significant test for comparison, followed by the Dunnet's "t" test, *$p$ <0.001, when the test and the standard were compared against the control, and the values were mean and SEM.

ANOVA was used as the statistically significant comparison test, with the Dunnet's t test (*$p < 0.001$) used to compare the results to the control. $Fe^{3+}$ to $Fe^{2+}$ transition deduced via spectrophotometry.

ANOVA was used to perform a statistically significant comparison test, followed by a Dunnet's t test. The

**Table 8:** Effect of MESP, MEBR and EDTA on $Fe^{2+}/Fe^{3+}$ metal chelation

| *Iron : Drug* | *OD at 525 nm* | *% Chelation of $Fe^{2+}$* | *OD at 460 nm* | *%Chelation of $Fe^{3+}$* |
|---|---|---|---|---|
| 1:00(control) | 0.315 | 0 | 1.032 | 0 |
| 1:0.25 MESP | 0.244 | 25.26 ± 3.15* | 0.874 | 13.74 ± 1.32* |
| 1:0.5 MESP | 0.224 | 36.68 ± 3.62* | 0.745 | 22.25 ± 2.75* |
| 1:1 MESP | 0.216 | 38.42 ± 2.55* | 0.732 | 29.25 ± 2.89* |
| 1:2.5 MESP | 0.200 | 43.56 ± 2.64* | 0.623 | 31.64 ± 2.94* |
| 1:5 MESP | 0.194 | 52.62 ± 2.36* | 0.613 | 36.25 ± 3.45* |
| 1:10 MESP | 0.145 | 66.24 ± 3.26* | 0.442 | 55.75 ± 3.35* |
| 1:0.25 MEBR | 0.234 | 27.25 ± 2.74* | 0.924 | 15.75 ± 2.24* |
| 1:0.5 MEBR | 0.223 | 38.34 ± 2.25* | 0.824 | 24.25 ± 2.15* |
| 1:1 MEBR | 0.205 | 41.78 ± 2.68* | 0.808 | 30.21 ± 2.04* |
| 1:2.5 MEBR | 0.198 | 46.95 ± 2.51* | 0.732 | 34.97 ± 2.44* |
| 1:5 MEBR | 0.188 | 53.78 ± 2.35* | 0.715 | 37.86 ± 2.35* |
| 1:10 MEBR | 0.152 | 67.95 ± 2.16* | 0.543 | 57.96 ± 3.74* |
| (1:10)Standard (EDTA) | 0.067 | 78.62 ± 4.27* | 0.145 | 85.43 ± 3.38* |

**Table 9:** Inhibition of lipid peroxidation –induction by $CCl_4$ system of MESP, MEBR and Vitamin-E

| S. No | Concentration (µg/mL) | %Inhibition |
|---|---|---|
| 1 | Control | - |
| 2 | MESP (25) | 22.43 ± 2.48* |
| 3 | MESP (50) | 39.64 ± 2.62* |
| 4 | MESP (100) | 43.45 ± 2.84* |
| 5 | MESP (200) | 52.74 ± 2.54* |
| 6 | MESP (300) | 61.25 ± 3.54* |
| 7 | MEBR (25) | 21.25 ± 3.56* |
| 8 | MEBR (50) | 38.25 ± 3.45* |
| 9 | MEBR (100) | 41.74 ± 2.84* |
| 10 | MEBR (200) | 48.35 ± 2.14* |
| 11 | MEBR (300) | 59.25 ± 2.48* |
| 12 | Standard (Vit E) | 67.24 ± 2.56* |

Statistical significant test for comparison was done by ANOVA, followed by Dunnet 's 't' test (n=6), *$p$< 0.001, when test and standard are compared against control, Values are Mean ± SEM.

**Table 10:** Inhibitory test on protein oxidative modification of MESP, MEBR and Vitamin-E

| *S. No* | *Concentration (µg/mL)* | *% inhibition* | *$IC_{50}$ Value (µg/mL)* |
|---|---|---|---|
| 1 | MESP (100) | 22.43 ± 2.36 | 426.35 ± 4.56 |
| 2 | MESP (200) | 43.22 ± 2.51 | |
| 3 | MESP (400) | 56.96 ± 2.64 | |
| 4 | MESP (600) | 67.33 ± 3.76 | |
| 5 | MESP (800) | 73.26 ± 3.37 | |
| 6 | MESP (1000) | 81.66 ± 3.78 | |
| 7 | MEBR (100) | 23.21 ± 2.36 | 430.16 ± 7.62 |
| 8 | MEBR (200) | 44.45 ± 3.48 | |
| 10 | MEBR (400) | 56.75 ± 6.47 | |
| 11 | MEBR (600) | 63.26 ± 8.64 | |
| 12 | MEBR (800) | 75.85 ± 3.79 | |
| 13 | MEBR (1000) | 82.34 ± 1.45 | |
| 14 | Standard (Vitamin-E100) | 32.54 ± 2.85 | 264.53 ± 5.94 |
| 15 | Vitamin-E(200) | 51.24 ± 2.96 | |
| 16 | Vitamin-E(400) | 63.54 ± 2.96 | |
| 17 | Vitamin-E(600) | 72.18 ± 3.54 | |
| 18 | Vitamin-E(800) | 80.65 ± 3.69 | |
| 19 | Vitamin-E(1000) | 81.55 ± 3.84 | |

concentrations of $Fe^{2+}$ and $Fe^{3+}$ were measured using the $Fe^{2+}$-dipyridyl complex (525 nm) and the $Fe^{3+}$-thiocynate complex (460 nm), respectively. When test and standard are compared to control, EDTA (ethylene diamine tetraacetic acid) is used as the control.

## Discussion

Medicinal plants are an affluent source of phytochemical compounds that can play a vital role to treat several chronic diseases.[24] An extensive number of potent biomolecules come from a diverse number of medicinal plants in recent times.[25] Scientists believed that these potent chemical constituents obtained from nature are used for treating many disorders with fewer side effects.[26] These potent compounds are highly capable of inhibiting the harmless act of a multiple number of chronic diseases.[27] Few chronic diseases are so critical and there are no specific drugs for those diseases.[28] In such cases, medicinal plants should be applied and they give an effective result in pharmacologically and phytochemically.[29]

In the present study, the investigation of ethyl acetate and methanol extracts from leaves and methanolic extraction of *S. perfoliata* and *B. retusa* revealed the presence of various phytoconstituents, including flavonoids, carbohydrates, cardiac glycosides, tannins, phenols, amino acids, alkaloids, steroids, proteins and terpenoids. These

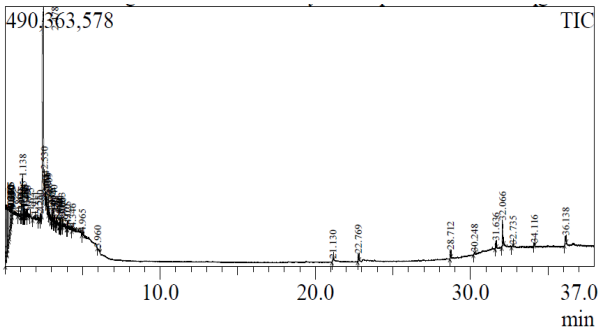



**Fig. 1:** GC-MS chromatogram of Methanolic extract of Smilax Perfoliata (MESP).

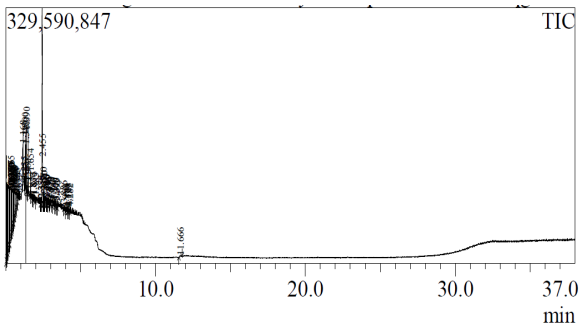



**Fig. 2:** GC-MS chromatogram of Methanolic extract of Breynia Retusa (MEBR).

bioactive phytoconstituents could be responsible for the therapeutic ability of various extracts of S. *perfoliata*. The analysis was carried out by gas chromatography-mass spectrometry (GC–MS), one of the most widely used techniques for separation of phytoconstituents. The GC–MS investigation of S. *perfoliata* extract revealed the presence of 26 phytochemical compounds, which could contribute to the medicinal properties of this plant species. They are 2-nonynoic acid, propanimidamide, N-(1-chloro-1-propenyl)-, monohydrochloride, 5-bromo-8-(5-nitrosalicylideneamino) quinoline hydrochloride, methylene chloride, nitrogen fluoride (N2F2), (Z)-, 20 1.426, 2,4,6-cycloheptatrien-1-one, nitrosyl chloride, hexahydro-isobenzofuran-1-one, ethene, 1-(2-methoxy-,3,5-dibromophenyl)-2-nitro-,cyclohexane, 1-methyl-2-propyl, trans-2,4-dimethylthiane, S,S-dioxide, 5-undecene, 8-methyl-, (E)- cycloheptane, methyl-, 3-hepten-2-one, (E)-, cyclohexane, (1,2-dimethylbutyl)-, 1-methyl-2-methylenecyclohexane, cyclopentane, 1-methyl-3-(1-methylethyl)- ethane, 1-chloro-1-fluoro, N-benzyl-N'-(1-benzylamino-2,2,2-trichloroethyl)-p-tolylcarboxamidine, n-hexadecanoic acid, 1,5,9-undecatriene, 2,6,10-trimethyl-, (Z)-cholesta-22,24-dien-5-ol, 4,4-dimethyl, 5-Cholestene-3-ol, 24-methyl-, 8,11-octadecadiynoic acid, methyl ester, undecane, 1,2-dibromo-2-methyl-, 9-(3,3-Dimethyloxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-ol, in that tropone or 2,4,6-cycloheptatrien-1-one is an organic compound with some importance in organic chemistry as a non-benzenoid aromatic.[30] The compound consists of a ring of seven carbon atoms with three conjugated alkene groups and a ketone group. Undecane is a liquid alkane hydrocarbon with the chemical formula $CH_3$ ($CH_2$) $9CH_3$. It is used as a mild sex attractant for various types of moths and cockroaches, and an alert signal for a variety of ants. It has 159 isomers.

The GC–MS investigation of *B. retusa* extract revealed the presence of 14 phytochemical compounds, they are acetyl bromide, N,N'-ethylenebis (N-nitroacetamide) 2-propanone, 1,1-dichloro-, methane, chlorotrinitro, 2-mercaptoethyl ether, silanamine, N-phenyl-, dodecane, 1-fluoro-, ethane, 1-chloro-1-fluoro-, L-methioninol, boranamine, 1-ethyl-N,N-dimethyl-1-(1-methyl-2-propenyl)-, lactonitrile, 3-chloro-, acetate hydrazinecarboximidamide, nitrate, 1H-1,2,3-triazole-4-carboxylic acid, 4,5-dihydro-1-phenyl-, phosphoric acid and bis(trimethylsilyl)monomethyl ester in that phenylsilane, also known as silylbenzene, a colorless liquid, is one of the simplest organosilanes with the formula $C_6H_5SiH_3$. It is structurally related to toluene, with a silyl group replacing the methyl group. Both of these compounds have similar densities and boiling points due to these similarities. Phenylsilane is soluble in organic solvents, and an essential amino acid in humans. As the precursor of other amino acids such as cysteine and taurine, versatile compounds such as SAM-e, and the important antioxidant glutathione, methionine plays a critical role in the metabolism and health of many species, including humans. It is encoded by the codon AUG and L-methioninol is also an important part of angiogenesis, the growth of new blood vessels. Supplementation may benefit those suffering from copper poisoning. Overconsumption of methionine, the methyl group donor in DNA methylation, is related to cancer growth in a number of studies,[31,32] and actonitrile is obtained by the addition of hydrogen cyanide to acetaldehyde. Lactonitrile is used in making esters of lactic acid.

Metal chelating capacity was significant as they reduced the concentration of the catalyzing transition metal in lipid peroxidation (Duh PD et al., 1999). Through epidemiological studies, it was reported that phenolic compounds have been shown to act as natural antioxidants by helping to neutralize free radicals and as metal chelating agents.[32] Antioxidants inhibit interaction between metal and lipid through formation of insoluble metal complexes with ferrous ion. Hence the data obtained for S. *perfoliata* and *B. retusa* reveals that some of the extracts demonstrate an effective capacity for iron binding, suggesting that its action as antioxidant may be related to its iron binding capacity that will prevent the free radical generation through fenton reaction.

Superoxide radical is known to be a very harmful species to cellular components as a precursor of more reactive oxygen species. The superoxide radical is known to be