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Identification of bioactive compounds by GCMS, quantification of total alkaloid, flavonoid, phenols and evaluation of anti-inflammatory activity in extracts of *Firmiana colorata*

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Abstract
Background: *Firmiana colorata*, a deciduous tree, is used to treat digestive ailments, intestinal dysfunction, general weakness and jaundice in traditional medicine. It is also known to possess antimicrobial properties against various microorganisms.
Objective: To identify bioactive compounds, to determine the total alkaloid, flavonoid, phenolic content and to evaluate anti-inflammatory activity in leaf, bark, and flower extracts of *Firmiana colorata*. The main challenge of the study was to identify as many as possible bioactive compounds of *F. colorata* as there is scanty literature available regarding it. Attempt was also made to find out the application of these compounds as sources of healing, treatment and substitutes for prevalent synthetic drugs available in the market.
Methods: Gas Chromatography-Mass Spectrometry was employed to identify bioactive compounds present in leaf, leaf oil, bark and flower extracts of *F. colorata*. Spectrophotometric method was used to quantitatively assess ethyl acetate and ethanol extracts of leaves, bark and flowers of *F. colorata* for their total alkaloid, flavonoid and phenolic content. AAPH-induced anti-hemolytic assay, LDH assay and protein denaturation assay were performed to determine the anti-inflammatory potential in the extracts of *F. colorata*.
Results: GC-MS analysis revealed the presence of 49 pharmacologically important bioactive compounds in leaf, leaf oil, bark and flower extracts of *F. colorata.* Quantitative analysis showed the highest phenolic and flavonoid content in ethyl acetate bark extract when compared to leaf and flower extracts. Anti-inflammatory activity was highest in ethanol flower extract. The study has revealed the distribution of various bioactive compounds across the different parts of the selected plant species and its potent anti-inflammatory activity contributes to the objective of the study.
Conclusion: The presence of several pharmacologically important bioactive compounds and potent anti-inflammatory activity exhibited by the extracts of *Firmiana colorata* justifies its use in traditional medicine and renders medicinal status to the plant.

Keywords: Anti-inflammatory activity, bioactive compounds, Clevenger apparatus, *Firmiana colorata*, GC-MS

Introduction

Herbal plants are used as sources of medicine by mankind since time immemorial. Around 80% of the world's population is reportedly dependent on traditional medicine today [1]. It has been hypothesized that the pharmacological properties of plants are due to the variety of phytochemicals present in them [2]. Phytochemicals are an overwhelming variety of organic compounds that vary remarkably in their qualitative and quantitative distribution along different parts of the plant, species and geographical area. They form an integrated part of the defence system of plants and therefore, most of these constituents are potent bioactive compounds [3].

Pharmaceutical industries are using bioactive compounds for the synthesis of useful drugs and as potent agents in the treatment of many diseases. Hence studies on pharmacological activities of these bioactive compounds have received wide attention in the scientific community. World Health Organisation has also emphasized the need to ensure the quality of medicinal plant products using modern controlled techniques and suitable standards [4].

Consequently, the identification and quantification of bioactive constituents in plants is the need of the day. Furthermore, methods used in the identification of bioactive compounds should be simple, reliable and repeatable. The screening of medicinal plants by spectrophotometric and chromatographic techniques has proven extremely useful in providing basic information on the phytochemical makeup of the plant. And, Gas Chromatography-Mass Spectrometry (GC-MS) has become a firmly established, compatible, reliable and commonly used key technique for phytochemical profiling of plant species [5].

Gas chromatography-mass spectrometry (GC–MS) is one of the fastest and most accurate techniques to detect various compounds including alcohols, long-chain hydrocarbons, esters, amino acids alkaloids, nitro compounds, organic acids and steroids. The best advantage of the technique is that it requires small volumes of plant extracts and identifies compounds in a single step using a mass detector and GC–MS libraries [6]. The most important bioactive compounds of plants identified by GCMS, analysed and intensively studied are alkaloids, flavonoids, tannins, glycosides and phenolic compounds. These bioactive compounds possess numerous health effects such as analgesic, antiseptic, antimicrobial, antioxidant, antithrombotic, vasodilatory activities and many more. Studies have also inferred the beneficial facet of plant-based drugs as good sources of anti-inflammatory agents [7].

Plant-based drugs are preferred owing to the fact that non-steroidal anti-inflammatory drugs (NSAID) used to treat and manage inflammation in contemporary medicine have not been therapeutically successful in all conditions. Moreover, adverse side effects associated with NSAIDs can lead to haemorrhage and gastric ulcers [8]. Therefore, screening indigenous plants for developing anti-inflammatory drugs is gaining momentum. Plant extracts used to reduce inflammation are characterized by either an overproduction of inflammatory mediators such as Reactive Oxygen Species (ROS), nitric oxide, interleukin-6 and pro-inflammatory cytokines or downregulation of pro-inflammatory cytokines, cyclooxygenases, reduced translocation of NF-kB to the nucleus and suppression of the nuclear factor-kappa B (NF-kB) signaling pathway. Plant extracts have also exhibited anti-inflammatory effects by interfering with lipopolysaccharide binding to Toll-like receptor 4 (TLR4), signaling and activating the antioxidative nuclear factor erythroid 2-related factor 2 (Nrt2) [9].

Oils have also been commonly used in traditional medicine to treat and manage inflammation and pain. They are used generally for topical application (often combined with massages). Essential oils are composed of low molecular weight volatile compounds which are mainly responsible for their distinct aroma and therapeutic activities. Essential oils can be extracted through hydro distillation from herbs, shrubs and leaves, flowers, fruits, roots, seeds and bark of trees [10]. Leaves of a deciduous tree, *Firmiana colorata*, were used in the current study to extract oil and identify the bioactive compounds present in it by GCMS.

Firmiana colorata belongs to the *Malvaceae* family and genus *Firmiana*. Most of the *Firmiana* species are grown for their attractively shaped flowers [11]. Furthermore, *Firmiana* also finds a place in traditional medicine and several species of *Firmiana* were observed to possess antimicrobial, anti-inflammatory, anticancer, neuroprotective and hepatoprotective properties. One of its species is *Firmiana colorata*, used in traditional medicine to treat digestive ailments, jaundice, general weakness, fever and intestinal dysfunction. *Firmiana colorata* is known to possess antimicrobial properties against several microorganisms. (12)

Despite the ethnobotanical importance of *F. colorata*, adequate studies on its anti-inflammatory properties, qualitative and quantitative accounts of phytochemicals are not available. The present study aimed to analyse leaf, leaf oil, bark and flower extracts of *F. colorata* through gas chromatography-mass spectrometry (GC-MS) to elucidate their chemical composition and to determine their potential applications. An effort is also being made to investigate the *In vitro* anti-inflammatory activities in ethyl acetate and ethanol extracts of leaves, bark and flowers of *F. colorata*. The present study can provide substantial information about the bioactive compounds of *F. colorata* that may further be utilized in the pharmacological industries.

Materials and Methods

Preparation of Plant extracts

Firmiana colorata leaves, bark and flowers were collected from the Indian Institute of Science, Bengaluru, India (geographical coordinates: 13° 1' 1" North, 77° 34' 1" East) and authenticated from Central Ayurveda Research Institute, Ministry of Ayush, Bengaluru, India. The voucher specimen number provided was SMPU/CARI/BNG/202021/1052. Collected samples were washed under running tap water and shade-dried for seven days at room temperature and ground into a coarse powder. *Firmiana colorata* leaf, bark and flower crude extracts (hereafter referred to as LE, BE, FE prefixed with EA- ethyl acetate or Eth- ethanol) were obtained by hot percolation extraction method using a Soxhlet apparatus. Sample powder weighed about 150 grams was packed in a Soxhlet apparatus and extracted with ethyl acetate as the first solvent followed by ethanol. A rotary evaporator was used to concentrate the filtrate under vacuum. A total of 6 extracts *viz*., EA-LE, EA-BE, EA-FE, Eth-LE, Eth-BE and Eth-FE were obtained using Soxhlet apparatus. All six extracts were used for the determination of TAC, TPC, TFC, AAPH-induced anti-haemolytic assay, lactate dehydrogenase assay and protein denaturation assay.

Determination of total phenolic content (TPC)

Folin–Ciocalteu (FC) method by spectrophotometry was employed to determine the total phenolic content in ethyl acetate and ethanol LE, BE and FE (1mg/ml) of *F. colorata*. l ml of each extract was taken in respectively labelled tubes followed by addition of 2ml of reaction mixture. FC reagent and 35% Na_2CO_3 in a ratio of 1:1 was combined to make the reaction mixture. All tubes were incubated for 45 minutes at room temperature. The optical density of the test was measured against a reaction mixture blank at 760 nm spectrophotometrically. TPC in each extract was determined using a calibration curve of standard gallic acid solution generated. The results are provided as micrograms of gallic acid equivalent per milligram of extract [13].

Determination of total flavonoid content (TFC)

The total flavonoid content in ethyl acetate and ethanol LE, BE and FE of *F. colorata* was determined by the aluminium chloride method by spectrophotometry. 1ml of LE, BE and FE (1mg/1 ml) were taken in three different tubes. 0.3 mL of 5% $NaNO_2$ was added to all the tubes, and incubated for 5 min at 25 °C followed by the addition of 10% methanolic solution of aluminium trichloride and 2 ml of 1N NaOH. After mixing thoroughly, the mixture was incubated for 15 minutes at room temperature. The absorbance of the test against the methanol blank was measured at 510 nm using a spectrophotometer. A calibration curve for standard rutin solution was generated and TFC was calculated in each extract. Results are represented as micrograms of rutin equivalent per milligram of extract [14].

Determination of Total Alkaloid content (TAC)
Bromocresol green (BCG) method by spectrophotometry was used to determine the total alkaloid content in ethyl acetate and ethanol LE, BE and FE of *F. colorata*. 1ml of each extract (1 mg/ml) was taken in respective tubes followed by addition the of 5 ml BCG solution and 5 ml phosphate buffer (pH 4.7). The mixture was transferred to a separating funnel and thoroughly agitated. The resultant complex was extracted using chloroform and the optical density of the complex was measured at 470 nm. A calibration curve for standard caffeic acid generated was used to determine TAC in each extract and results are provided as micrograms of caffeic acid equivalent per milligram of extract [15].

Extraction of essential oil from leaves of *Firmiana colorata*
Leaves of *Firmiana colorata* were washed, dried and ground to coarse powder. 200 grams of leaf powder was subjected to hydro distillation for 6 hours using a Clevenger apparatus with a graduated column to enable the measurement of oil yield directly. Anhydrous sodium sulphate was added to remove moisture content in it. The yield was calculated in % volume/gram of leaf powder. The oil samples were stored in air-tight containers and refrigerated at < 4^0C until GC-MS analysis [16].

Identification of volatile compounds by GC–MS Analysis
GC-MS analysis was performed to determine the bioactive compounds in leaf, leaf oil, bark and flower extracts of *Firmiana colorata*. The GC-MS system was equipped with a Perkin Elmer Elite-5MS column (30 m length × 0.25 mm internal diameter × 1 micron in thickness). The carrier gas helium was maintained at a flow rate of 2ml/min and the split ratio was 10:1. The initial temperature of the GC oven was operated at 80 ^{0}C with 2-minute initial hold followed by a ramp:1 increase of 10 ^{0}C/min up to 150 ^{0}C with a hold time of 1.00 minute and ramp:2 increase of 15 ^{0}C/min up to a final temperature of 250 ^{0}C with a total hold time of 25.00 minutes for leaf oil, LE and BE. Whereas the total hold time for FE was 45 minutes. The samples were ionized with 70 eV GCMS radiation. The input temperature was 250 °C and the source temperature was 230 °C. The mass of the compounds was scanned from 29 to 800 amu. Identification of volatiles was performed by comparing their mass spectra with those of NIST/EPA/NIH Mass Spectral Library 2014 coupled with the GC-MS system.

Determination of Anti-inflammatory activity
Preparation of Erythrocyte Suspension
Whole blood samples were collected from physiologically normal adult human subjects who had not taken NSAIDs for at least 7 days prior to the blood withdrawal. This was the prime criterion for the selection of human subjects. The blood samples were centrifuged at 3000 rpm for 10 minutes in heparinized centrifuge tubes to remove the plasma and separate packed red cells. It was washed thrice with normal saline. Blood volume was measured and reconstituted with 10 mM phosphate buffer solution (pH 7.4) to obtain 40% RBC suspension. (17) This was used as an erythrocyte stock suspension. 10% erythrocyte suspension was prepared from stock and further used for AAPH-induced anti-haemolytic assay and Lactate dehydrogenase assay.

a. Human red blood cell (HRBC) membrane stabilization assay
Haemolysis was induced in 10% erythrocyte suspension by adding an equal volume of 0.2 M AAPH [2,2′-Azobis (2-amidinopropane) dihydrochloride]. Different concentrations (0-100 µg/mL) of EA and Eth LE, BE and FE of *F. colorata* were immediately added to the reaction mixture taken in respective labelled tubes. Erythrocyte suspension served as a positive control, reaction mixture served as a negative control and 0.2% Triton (in PBS) with 100% haemolysis was taken as reference control. All tubes were incubated at 37 °C for 2 hours. 8 volumes of PBS were added to all tubes post-incubation for dilution and centrifuged at 2000 rpm for 10 min. The supernatant was collected and the degree of haemolysis was assessed by measuring the optical density at 540 nm. The percentage of haemolysis or membrane stabilization was calculated, and results were expressed as IC_{50} value in comparison with L-ascorbic acid as standard [18].

b. Lactate dehydrogenase (LDH) assay
The amount of LDH released by the damaged erythrocytes was calculated using the method described by Lenfant (2000) with slight modifications. 500 µl of 10% erythrocytes were incubated for 2 hours with an equal volume of 100 mM AAPH solution that contained EA and Eth - LE, BE and FE of *F. colorata* respectively. Whilst tubes without extracts served as a negative control and 40 µg/ml of L-Ascorbic acid served as a positive control. 200 µl of the reaction mixtures were diluted in 400 µl of PBS following the incubation period and centrifuged at 3000 rpm for 5 minutes. The amount of LDH released from 200 µl of supernatant was measured using an LDH estimation kit (Cayman Chemical - 601170, India) with the results represented as percent LDH released [19].

c. Protein denaturation assay
Effect of EA and Eth - LE, BE and FE of *F. colorata* extracts on inhibition of protein denaturation was determined as per the procedure described by Naz *et al.*, with some modifications. Different concentrations of each of the extracts (0-800 µg/ml) were taken in separate tubes, labelled promptly and mixed well with 1% BSA (bovine serum albumin). The pH of the reaction mixture was adjusted using a small amount of 1N HCl. The reaction mixture was incubated for 10 minutes at 37^0C and later placed in a water bath maintained at 70^0C for 5 minutes. The tubes were cooled at room temperature and turbidity was measured spectrophotometrically at 660 nm. (20) Diclofenac sodium was used as a reference drug. The assay was performed in triplicate. The percentage of inhibition of protein was determined with respect to control using the following formula.

$$\text{Percentage inhibition (\%)} = \frac{\text{absorbance of control} - \text{absorbance of test}}{\text{absorbance of control}} X100$$

Statistical Analysis

TAC, TPC and TFC spectrophotometric determinations were replicated thrice and quantitative results are reported as mean±standard deviation. HRBC membrane stabilization, Lactate dehydrogenase, Protein denaturation assays were also done in triplicates and results are reported as mean±standard deviation. Microsoft Excel 2019 was used for all statistical and graphical evaluations of results.

Results and Discussion

Several biological activities have been reported on genus *Firmiana*, *viz*; antidiabetic, antimicrobial, antioxidant, anticancer, anti-inflammatory and others. (21, 22, 23) On the other hand, a review of current literature has traced less information dealing with the biological activities of the selected plant species *Firmiana colorata*. Limited data available on *Firmiana colorata* has revealed that the seed oil of *Firmiana colorata contains* Cyclopropenoid fatty acids including sterculic, malvalic, myristic, palmitic, stearic, oleic and linoleic acids. (24) Two new coumarin derivatives isolated from *Firmiana colorata* namely 4-(3,5-dihydroxyphenyl)-3,5,7-trihydroxy-2H-chromen-2-one and 4-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-(((2R,3S,4R,5R,6S)-3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl) oxy)-2H-chromen-2-one with significant immunomodulatory and anticancer activities have been reported [25]. The data reported by Soumita Bhattacharjee et al. [26] and Alia Yassin Ragheb Abd Al-Rahman [27] on a few bioactive compounds present leaf extract of *F. colorata* may differ from the current study data. In view of the fact that biosynthesis and composition of bioactive compounds varies across different species and even within the same species due to geographical distribution of the plant, environmental conditions, abiotic constraints such as water, stress level of pollution, local climatic and seasonal factors [28].

The present study appears to be the first ever to identify the bioactive compounds present in the leaf, leaf oil, bark and flower extracts of *F. colorata* through GCMS. Evaluation of anti-inflammatory activity possessed by LE, BE and FE involving HRBC membrane stabilization assay, LDH assay and Protein denaturation assay is also reported for first time. The results of the study have been tabulated.

The concentration of TAC, TPC and TFC present in ethyl acetate and ethanol LE, BE and FE of *F. colorata* is given in Table 1 and presented in figures 1-3 which show that phenolic and flavonoid content was more in FE than BE and LE. Also, alkaloid content was higher in BE when compared to LE and FE. A similar study reports that leaf extract of *F. colorata* has high phenolic and flavonoid content [29]. While limited data is available on the alkaloid, flavonoid and phenolic content of *F. colorata*, there is sufficient data available for other species of *Firmiana* and their medicinal potency has been substantiated. In view of the same, alkaloids, flavonoids and phenolic content of *F. colorata* will presumably contribute to its anti-inflammatory activity.

The results of GCMS analysis of leaf oil, LE, BE and FE of *F. colorata* are given in Tables 2-5. 49 compounds were identified via GCMS. Retention time (RT), peak area (PA), molecular formula, molecular weight, hit spectra and known bioactivities of each compound have been tabulated and chromatograms incorporated as figures 4- 7.

Details of 11 compounds present in LE of *F. colorata* are provided in Table 2. LE contains pharmacologically important bioactive compounds which include Neophytadiene, Stearic acid, 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol, Palmitic acid, ethyl ester, Eicosanoic acid, ethyl ester and n-Hexadecanoic acid.

Table 3 provides the details of 23 compounds identified in leaf oil of *F. colorata*. The molecular weight of the compounds ranged from 214 to 604 g/mol. 2-Piperidinone, N-[4-bromo-n-butyl], Tetradecane,2,6,10-trimethyl, Nonadecane, Phytol and 2-Hexyl-1-octanol are a few of them. Presence of such compounds presumably contributed to the anti-inflammatory effects exhibited by leaf oil. Tetracontane, 3,5,24-trimethyl, Carbonic acid, eicosyl vinyl ester, Heptadecane,2,6-dimetyl, 1-Decanol, 2-octyl-, Heptadecane,2,6-dimetyl, Silane, trichlorodocosyl- have also been identified but bioactivity of these compounds has not been reported in the literature. There is scope for further research on these compounds to develop new drugs.

8 compounds *viz*., Pterine-6-carboxylic acid, Spiro [Androst-5-Ene-17,1'- Cyclobutan]-2'-One, 3-Hydroxy-, (3.β,17β)-, 9-Octadecenoic acid(Z)-, phenyl methyl ester, Panaxjapyne A, Nonadecane, β Carotene, 1-Iodo-2-methylundecane were present in the bark extract of *F. colorata* (table 4). Majority of the compounds identified in BE have been reported to possess medicinal properties.

FE showed the presence of Ribitol,1,3:2,4-di-O-benzylidene-, Santochlor, Undecane, Ribitol, 1,3:2,4-di-O-benzylidene-, Palmitic acid, methyl ester, 10-Octadecenoic acid, methyl ester and Lauric acid, methyl ester as mentioned in table 5. Most important among them is Palmitic acid, methyl ester which is known to inhibit neuroinflammation [30].

Among the 49 identified compounds, Neophytadiene, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Stearic acid, Eicosanoic acid, ethyl ester, 2-Piperidinone, N-[4-bromo-n-butyl]-, Tetradecane,2,6,10-trimethyl, Spiro[Androst-5-Ene-17,1'- Cyclobutan]-2'-One, 3- hydroxy-, (3.β,17β)-, 9-Octadecenoic acid(Z)-,phenyl methyl ester, Phytol and Ribitol, 1,3:2,4-di-O-benzylidene- are known to possess potent anti-inflammatory activity (tables 2-5). These Phytochemicals may exhibit different mechanisms of anti-inflammatory effects. They may act on the three main inflammatory signaling pathways *viz*., NF-κB, STATs and MAPKs which in turn play crucial roles in inducing, promoting and regulating inflammatory responses in the body [31]. Additionally, RBC membrane is similar to the lysosomal membrane, inhibition of RBC haemolysis may provide insights into the inflammatory process. Stabilization of these cell membranes may retard the lysis and subsequent release of the cytoplasmic contents which minimizes the tissue damage and hence the inflammatory response [32]. Therefore, substances that contribute significant protection to cell membranes against injurious substances are important in the event of inhibiting inflammation. Further, it is possible that plant extracts may affect the ratio of surface area/volume of the cells by expansion of membrane or the shrinkage of cells and interaction with membrane proteins [33]. Therapeutic strategies targeting these inflammatory signaling pathways may be used to treat inflammatory diseases and various phytochemicals modulating different inflammatory cascades can be exploited for adjunctive therapy.

The bioactivity of several identified compounds has not been reported in the literature. It is presumed that these compounds have medicinal potency on the grounds of the antimicrobial, anti-haemolytic, anti-inflammatory and antioxidant properties exhibited by *Firmiana colorata*. Hence, isolation and in-depth study of these compounds is necessary to identify their biological activities to enable the discovery of a new class of anti-inflammatory agents and other novel drugs.

The results of HRBC membrane stabilization, LDH and Protein denaturation assays are given in Tables 6 and 7 and figures are also provided (figures 8.8 - 8.9). The anti-haemolytic activity was found to be high and the release of LDH was least in ethyl acetate and ethanol flower extracts. LE showed the least anti-haemolytic activity and ethyl acetate bark extract had moderate anti-inflammatory activity. Inhibition of protein denaturation was also exhibited more by flower extract (figure 8.10). Overall, anti-inflammatory activity was more in flower extracts of *F. colorata* and it may be attributed to Ribitol,1,3:2,4-di-O-benzylidene-. It could also be due to Cyclopentane tridecanoic acid, methyl ester, which lacks data on its bioactivity.

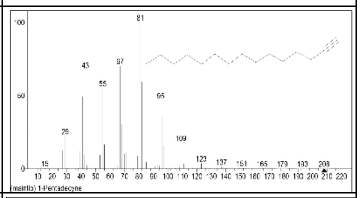
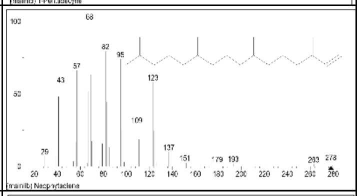
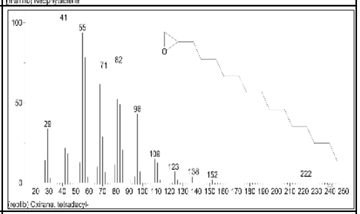
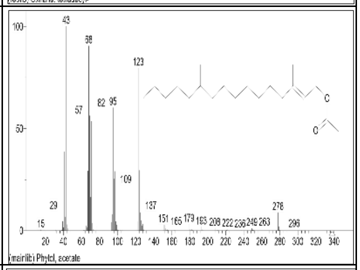
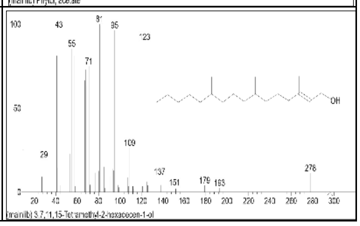
Results of the present study clearly demonstrate that extracts of *Firmiana colorata* have potent anti-inflammatory activities owing to the considerable quality and quantity of phytochemicals present in them. Also, GC-MS analysis is the stepping stone toward understanding the nature of bioactive compounds present in *F. colorata*. Further detailed studies on these compounds may throw light on the mechanism of actions at the molecular level and substantiate the results of the bioactivity profile of *F. colorata*.

Table 1: Total phenol, flavonoid and alkaloid content in leaf, bark and flower extracts of *Firmiana colorata*

	Total alkaloid content µg/mg extract (CAE)±SD		**Total phenolic content µg /mg extract (GAE)±SD**		**Total flavonoid content µg /mg extract (RE)±SD**	
	EA	**Eth**	**EA**	**Eth**	**EA**	**Eth**
LE	490±0.0182	730±0.049	460±0.0264	630±0.0508	260±0.0152	330±0.0264
BE	880±0.0193	775±0.0435	380±0.0763	430±0.0696	310±0.01	530±0.0935
FE	290±0.002	250±0.021	720±0.02	530±0.0132	300±0.0152	880±0.1721

CAE - caffeic acid equivalent, GAE - gallic acid equivalent, RE - rutin equivalent

Table 2: GC-MS analysis of leaf extract of *F. colorata*

Sl. No	**RT**	**PA%**	**Identified compound**	**MF**	**MW (g/mol)**	**Nature of compound**	**Bioactivity**	**Hit spectrum**
1	6.50	1.5	1-Pentadecyne	$C_{15}H_{28}$	208	long-chain alkyne	No activity reported	
2	8.71	2.9	Neophytadiene	$C_{20}H_{38}$	278	diterpene	Antipyretic, analgesic, anti-inflammatory, anti-microbial, antioxidant [34]	
3	11.73	0.2	Oxirane, tetradecyl-	$C_{16}H_{32}O$	240	oxygenated hydrocarbon	Antioxidant [35]	
4	12.39	1.4	Phytol, acetate	$C_{22}H_{42}O_2$	338	acyclic diterpenoids	No activity reported	
5	12.64	16.1	3,7,11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	diterpenoid	Antimicrobial, anti-inflammatory, antitubercular activity, antimycobacterial activity [36]	

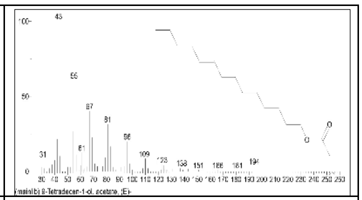
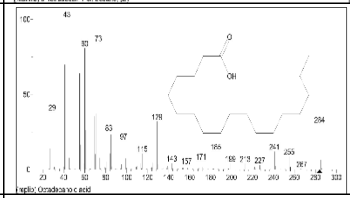
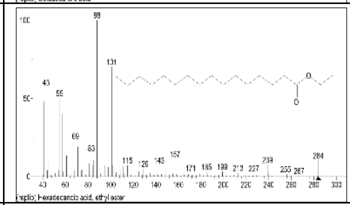
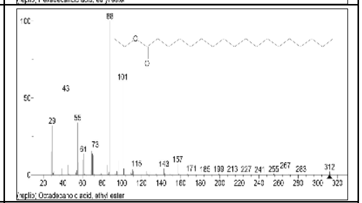
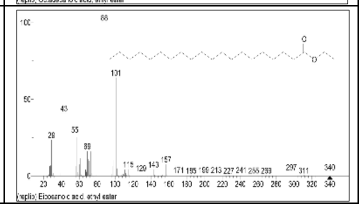
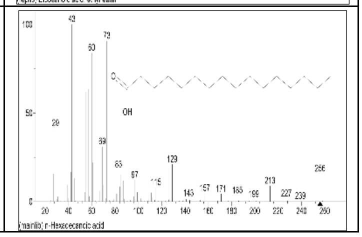
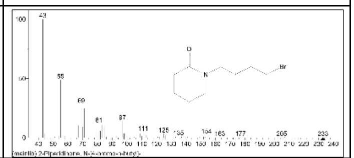
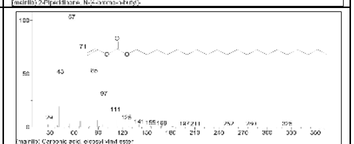
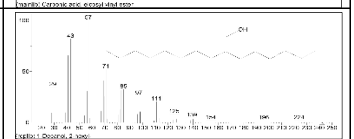
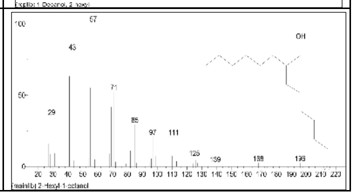
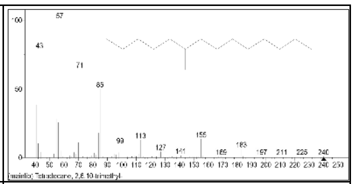
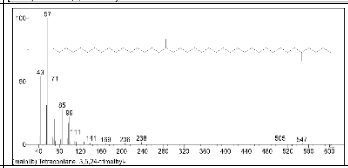
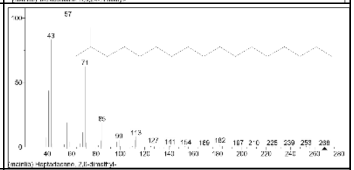
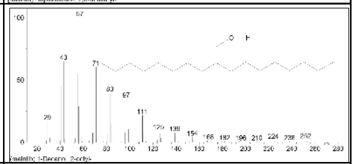
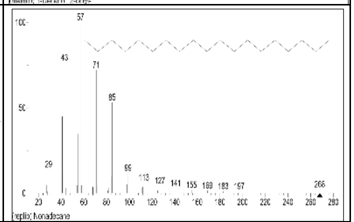
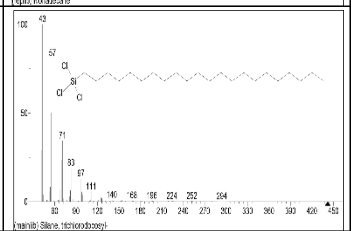
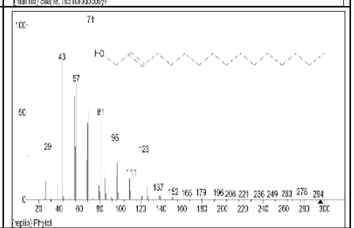
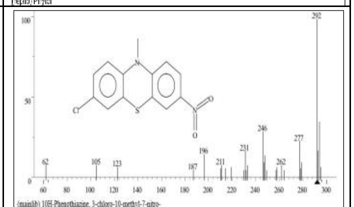
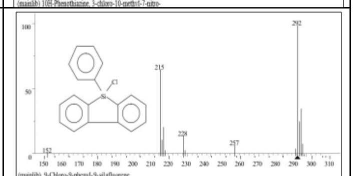
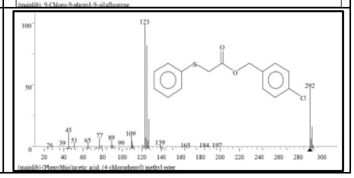
6	12.89	4.7	9-tetradecen-1-ol-acetate, (E)-	$C_{16}H_{30}O_2$	254	carboxylic ester	No activity reported	
7	13.72	24.1	Stearic acid	$C_{18}H_{36}O_2$	284	saturated fatty acid	Anti-inflammatory, antiandrogenic, cancer preventive, hypocholesterolaemia, 5- alpha reductase inhibitor [37]	
8	14.41	32.5	Palmitic acid, ethyl ester	$C_{18}H_{36}O_2$	284	saturated fatty acid	Antifungal, antitumour, antibacterial [38]	
9	14.60	5.7	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	octadecanoate ester	Antioxidant, anti-inflammatory [39]	
10	14.90	1.4	Eicosanoic acid, ethyl ester	$C_{22}H_{44}O_2$	340	saturated fatty acid	Anti-inflammatory, anti-thermogenic [40]	
11	18.77	10.7	n-Hexadecenoic acid	$C_{16}H_{32}O_2$	256	saturated fatty acid	Antioxidant hypocholesterolemic nematicide, antiandrogenic, hemolytic inhibitor, pesticide, lubricant, 5-Alpha reductase inhibitor, antipsychotic. [41]	

Table 3: GC-MS analysis of leaf oil of *F. colorata*

Sl. no	RT	PA%	Identified compound	MF	MW (g/mol)	Nature of compound	Bioactivity	Hit spectrum
1	10.74	0.24	2-Piperidinone, N-[4-bromo-n-butyl]-	$C_9H_{16}BrNO$	233	Alkaloid	Antimicrobial, anti-inflammatory [42]	
2	10.96	0.23	Carbonic acid, eicosyl vinyl ester	$C_{23}H_{44}O_3$	368	Acid diester	No activity reported	
3	11.01	1.37	1-Decanol,2-hexyl-	$C_{16}H_{34}O$	242	Aliphatic alcohol	Antimicrobial [43]	
4	11.27	3.46	2-Hexyl-1-octanol	$C_{14}H_{30}O$	214	Aliphatic alcohol	Anti-helminthic [44]	

5	11.62	5.62	Tetradecane,2,6,10-trimethyl	$C_{17}H_{36}$	240	Isoprenoid alkane	Anti-inflammatory, Antifungal [45]	
6	11.87	8.81	Tetracontane, 3,5,24-trimethyl	$C_{43}H_{88}$	604	Alkane	No activity reported	
7	12.02	14.3	Heptadecane,2,6-dimetyl	$C_{19}H_{40}$	268	alkane	No activity reported	
8	12.38	9.59	1-Decanol, 2-octyl-	$C_{18}H_{38}O$	270	Aliphatic alcohol	No activity reported	
9	12.67	16.81	Nonadecane	$C_{19}H_{40}$	268	Alkane	Anti-HIV, antioxidant, antibacterial, antifungal, antimalarial [46]	
10	12.38	7.46	Silane, trichlorodocosyl-	$C_{22}H_{45}Cl_3Si$	442	Organosilicon	No activity reported	
11	13.09	19.34	Phytol	$C_{20}H_{40}O$	296	Diterpene alcohol	Antinociceptive antioxidant, anti-inflammatory antiallergic, immunostimulant antimicrobial [47]	
12	13.32	19.01	10H-Phenothiazine, 3-chloro-10-methyl-7-nitro-	$C_{13}H_9CIN_2OS$	292.07	Phenothiazine derivative	No activity reported	
13	13.37	13.06	9-Chloro-9-phenyl-9-silafluorene	$C_{18}H_{13}ClSi$	292.04	-	No activity reported	
14	13.49	22.84	(Phenylthio) acetic acid, (4-chlorophenyl) methyl ester	$C_{15}H_{13}ClO_2S$	292.03	Methyl ester	No activity reported	

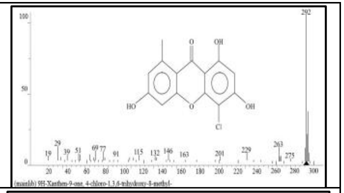
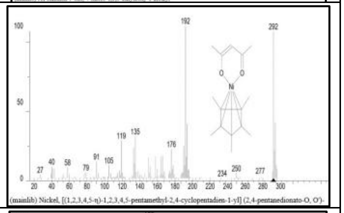
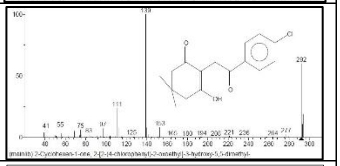
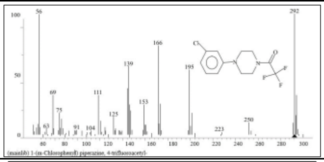
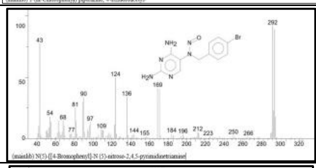
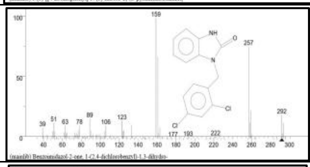
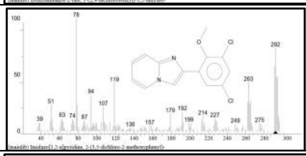
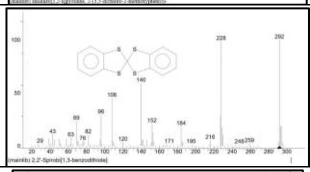
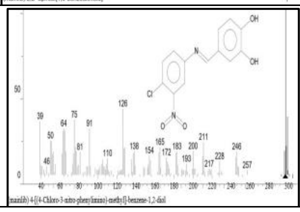
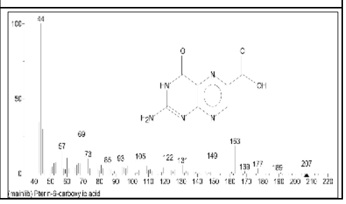
15	13.65	7.18	9H-Xanthen-9-one, 4-chloro-1,3,6-trihydroxy-8-methyl-	$C_{14}H_9ClO_5$	292.01	xanthone	No activity reported	
16	13.68	5.09	Nickel, [(1,2,3,4,5-η)-1,2,3,4,5-pentamethyl-2-4-cyclopentadien-1-yl] (2,4-pentanedionato-O,O′)-	$C_{15}H_{22}NiO_2$	292.09	-	No activity reported	
17	13.80	0.68	Cyclohexen-1-one, 2-[2-(4-chlorophenyl)-2-oxoethyl]-3-hydroxy-5,5-dimethyl	$C_{16}H_{17}ClO_3$	292.08	-	No activity reported	
18	13.84	0.39	1-(m-Chlorophenyl) piperazine, 4-trifluoroacetyl-	$C_{12}H_{12}ClF_3N_2O$	292.05	-	No activity reported	
19	13.98	2.73	N(5)-[4- Bromophenyl]-N(5)-nitroso-2,4,5-pyrimidinetriamine	$C_{11}H_{11}BrN_6O$	322	-	No activity reported	
20	14.12	2.85	Benzoimidazol-2-one, 1-(2,4-dichlorobenzyl)-1,3-dihydro	$C_{14}H_{10}ClN_2O$	292.01	-	No activity reported	
21	14.28	4.64	Imidazo[1,2-a] pyridine, 2-(3,5-dichloro-2-methoxyphenyl)-	$C_{14}H_{10}Cl_2NO$	292.01	-	No activity reported	
22	14.51	3.71	2,2′-Spirobi[1,3-benzodithiole]	$C_{13}H_8S_4$	291	-	No activity reported	
23	14.70	6.6	4-[(4-chloro-3-nitro-phenylimino)-methyl]-benzene-1,2-diol	$C_{13}H_9ClN_2O_4$	292.02	-	No activity reported	

Table 4: GC-MS analysis of bark extract of *F. colorata*

Sl no	RT	PA%	Identified compound	MF	MW (g/mol)	Nature of compound	Bioactivity	Hit spectrum
1	12.37	3.4	Pterine-6-carboxylic acid	$C_7H_5N_5O_3$	207	Pterin carboxylates	Anti-cancer anti-viral [48]	

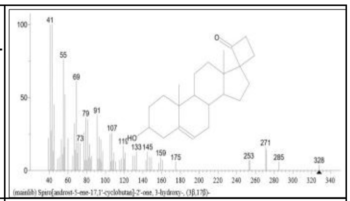
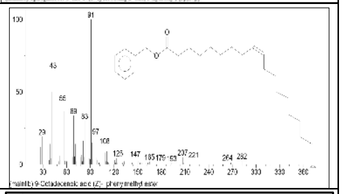
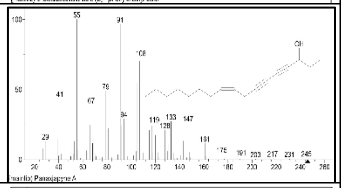
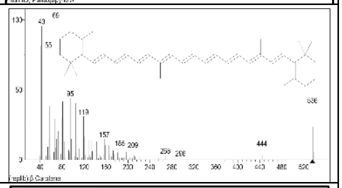
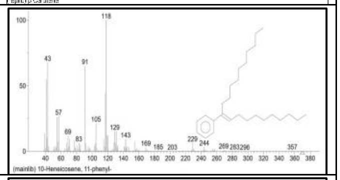
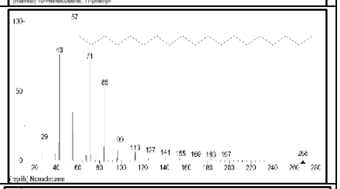
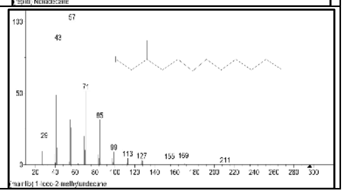
2	13.13	21.3	Spiro [Androst-5-Ene-17,1'- Cyclobutan]-2'-One, 3-Hydroxy-, (3.β,17β)-	$C_{22}H_{32}O_2$	328	Steroid	Antimicrobial, anticancer, anti-inflammatory, antiarthritic, antiasthma, antioxidant, hepatoprotective (49)	
3	13.95	1.1	9-Octadecenoic acid(Z)-,phenyl methyl ester	$C_{25}H_{40}O_2$	372	Oleic acid ester	Hypocholesterolemic5-alpha reductase inhibitor, anti-inflammatory (50)	
4	15.97	16.8	Panaxjapyne A	$C_{17}H_{26}O$	246	Polyenes	Anticancer, α-glucosidase inhibitors (51)	
5	16.03	17.1	β Carotene	$C_{40}H_{56}$	536	Terpenoid	Anti-ulcer, antioxidant (52)	
6	17.01	3.9	10-Heneicosene,11-phenyl-	$C_{27}H_{46}$	370	Saturated hydrocarbon	No activity reported	
7	18.61	27.2	Nonadecane	$C_{19}H_{40}$	268	Alkane	Anti-HIV Antioxidant, antibacterial, antifungal, antimalarial (53)	
8	20.90	9.2	1-Iodo-2-methylundecane	$C_{12}H_{25}I$	296	Iodoalkane	Antimicrobial, enhance reproductive activities (54)	

Table 5: GCMS analysis of flower extract of *F. colorata*

Sl no	RT	PA%	Identified compound	MF	MW (g/mol)	Nature of compound	Bioactivity
1	7.82	38.15	Ribitol, 1,3:2,4-di-O-benzylidene-	$C_{19}H_{20}O_5$	328	-	Activity against breast and colon cancer, antimicrobial, anti-carcinogenic, anti-inflammatory, antidiabetic [55]
2	12.29	7.16	Santochlor	$C_6H_4Cl_2$	146	Chlorophenol compound	Treatment of eye infection, insecticides
3	15.04	19.43	Undecane	$C_{11}H_{24}$	156	Alkane	Antimicrobial, transducer for immunosensor and its method for production, carcinogens and enzyme inhibitor [56]
4	26.66	6.44	Lauric acid, methyl ester	$C_{18}H_{36}O_2$	284	Fatty acid	Flavouring agent
5	31.29	6.21	Cyclopentane tridecanoic acid, methyl ester	$C_{19}H_{36}O_2$	296	Ester	No activity reported
6	35.49	18.36	Palmitic acid, methyl ester	$C_{17}H_{34}O_2$	270	Ester	Neuroprotective, inhibits cardiac arrest induced neuroinflammation and mitochondrial dysfunction
7	38.85	4.25	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	Unsaturated fatty Acid methyl ester	Antibacterial, antifungal, antioxidant, decrease blood cholesterol [57]

Table 6: Antihaemolytic and lactate dehydrogenase test effects of leaf, bark and flower extracts of *F. colorata*

	Haemolysis (IC_{50} µg/ml)		LDH release (%)	
	Ethyl acetate	Ethanol	Ethyl acetate	Ethanol
LE	69.51±2.59	62.68±4.64	36.54±1.46	38.85±1.97
BE	47.38±7.74	51.85±5.84	27.46±0.93	29.70±1.60
FE	44.82±7.78	44.73±6.77	26.02±1.75	24.92±1.41
Std	32.85±2.90		16.09±0.83	

Table 7: Percentage inhibition of protein denaturation exhibited by leaf, bark and flower extracts of *F. colorata*

Extracts	Percentage inhibition of protein denaturation (%)		
	at 200 µg/ml of extract	at 400 µg/ml of extract	at 800 µg/ml of extract
EA-LE	53.12±0.021	56.56±0.013	56.87±0.02
EA-BE	57.18±0.05	84.37±0.44	88.75±0.033
EA-FE	56.87±0.27	60.62±0.346	59.68±0.48
Eth-LE	34.06±0.301	43.75±0.021	47.81±0.333
Eth-BE	25±0.076	48.75±0.067	51.56±0.432
Eth-FE	69.06±0.024	81.25±0.731	83.75±0.372
Std-DS	89.06±0.044	90.31±0.051	91.25±0.323

Std-DS – standard diclofenac sodium

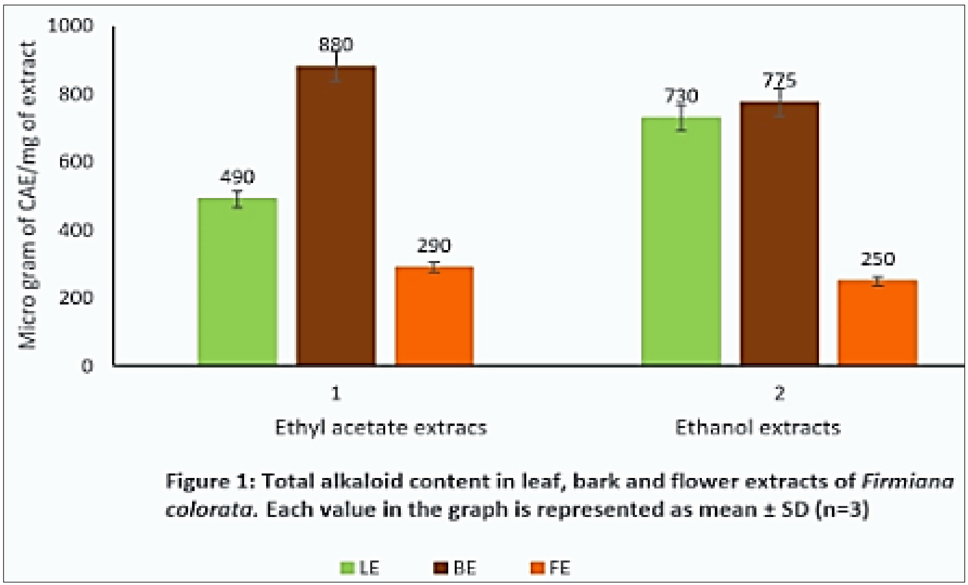


Figure 1: Total alkaloid content in leaf, bark and flower extracts of *Firmiana colorata*. Each value in the graph is represented as mean ± SD (n=3)

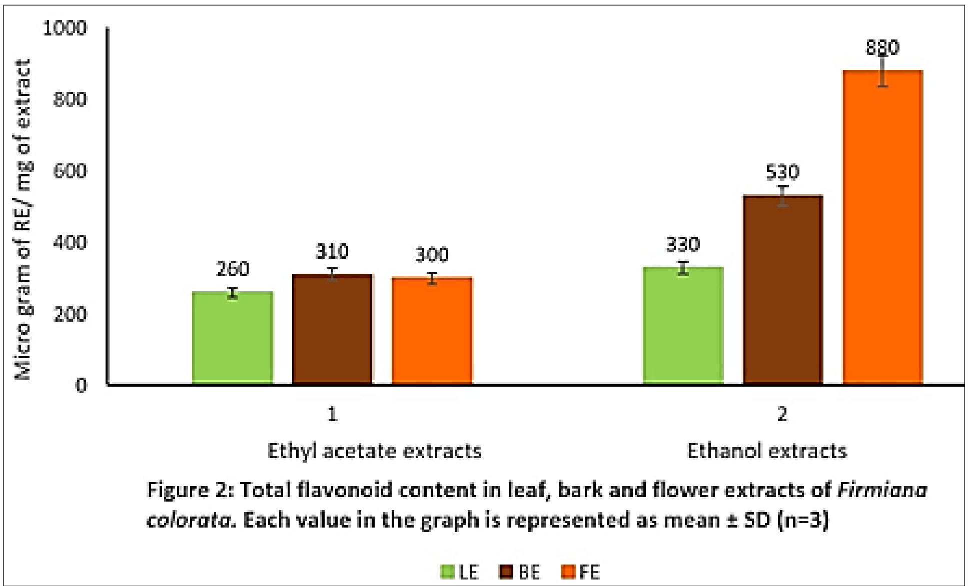


Figure 2: Total flavonoid content in leaf, bark and flower extracts of *Firmiana colorata*. Each value in the graph is represented as mean ± SD (n=3)

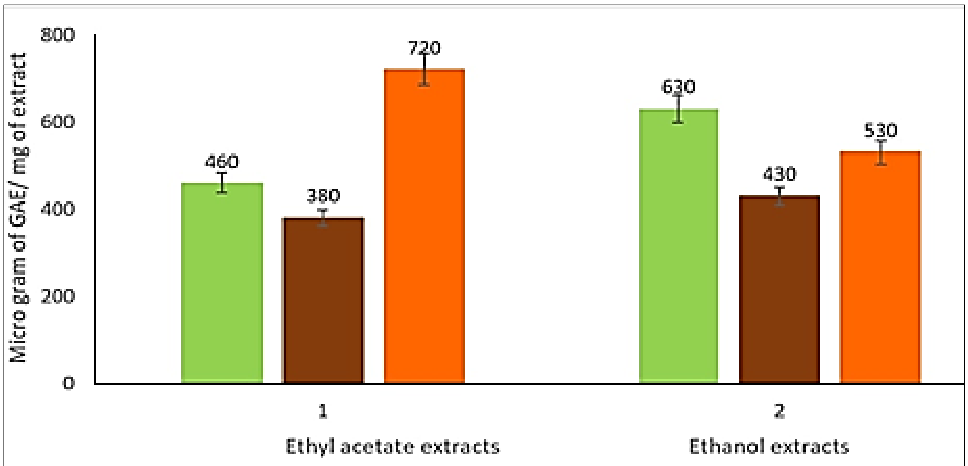


Figure 3: Total phenol content in leaf, bark and flower extracts of *Firmiana colorata*. Each value in the graph is represented as mean ± SD (n=3)

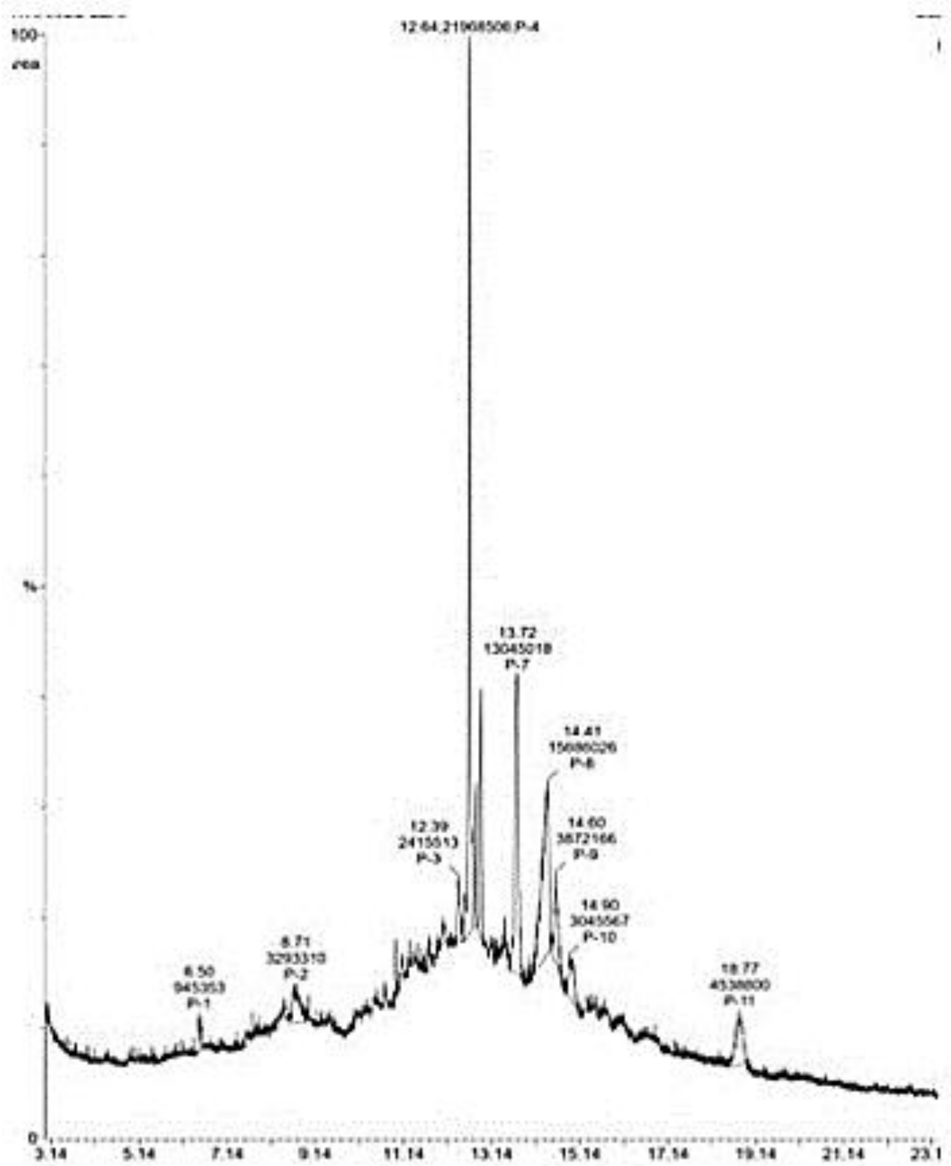


Fig 4: GCMS chromatogram-LE of *F. colorata*

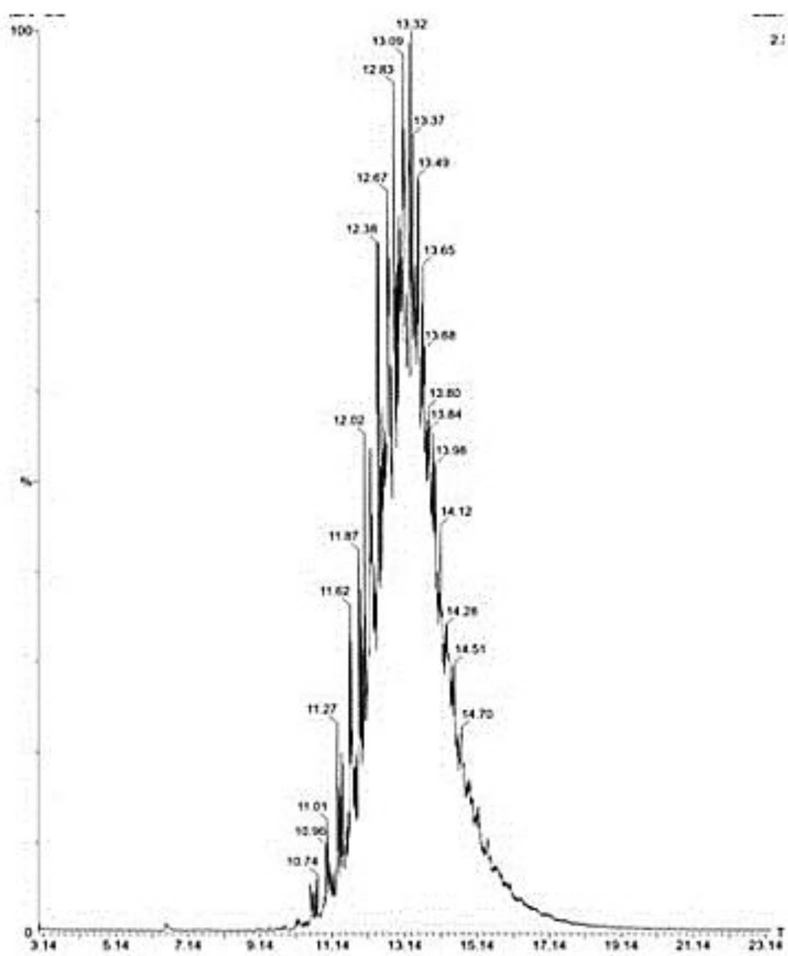


Fig 5: GCMS chromatogram-leaf oil *F. colorata*

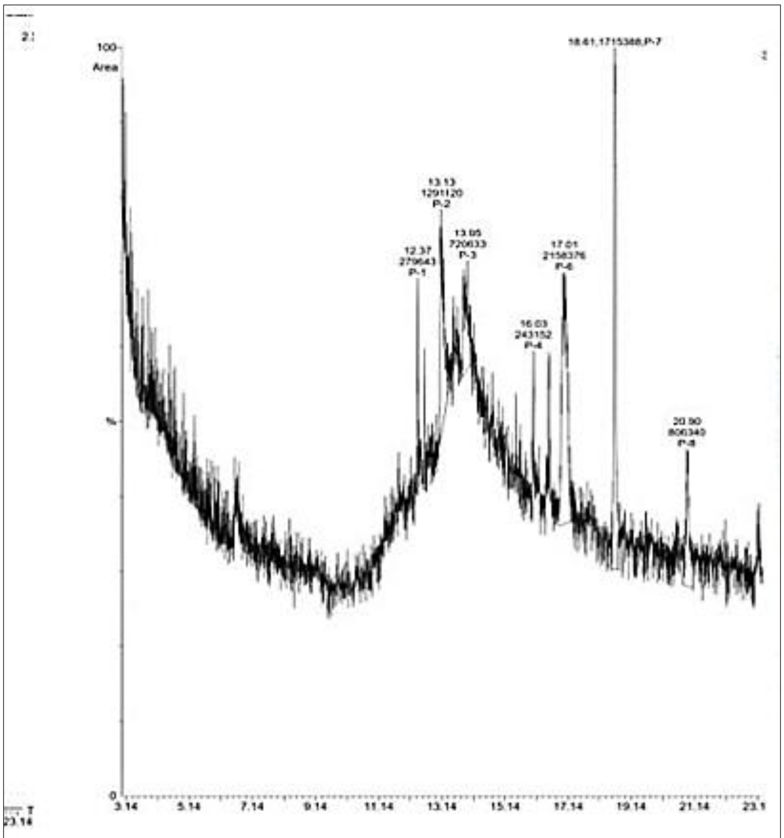


Fig 6: GCMS chromatogram-BE of *F. colorata*

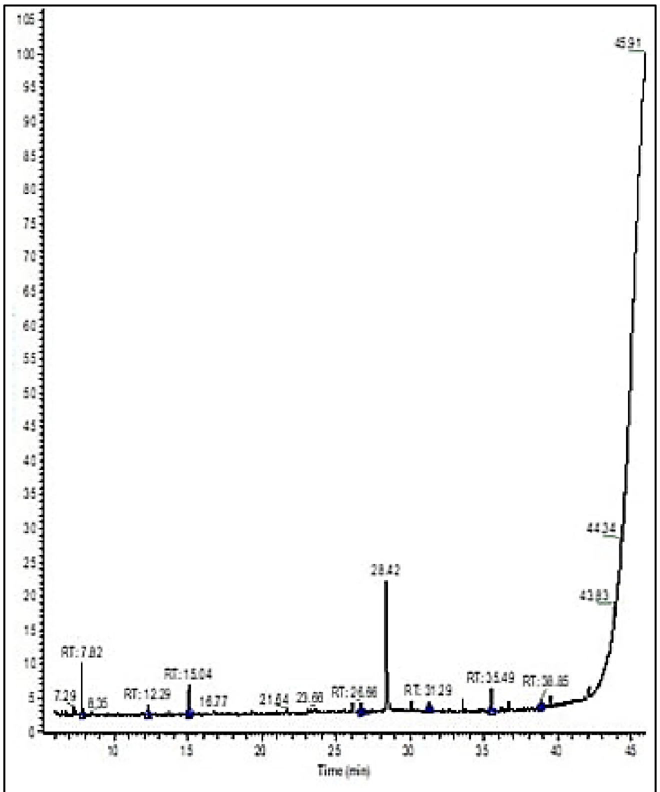


Fig 7: GCMS chromatogram-FE of *F. colorata*

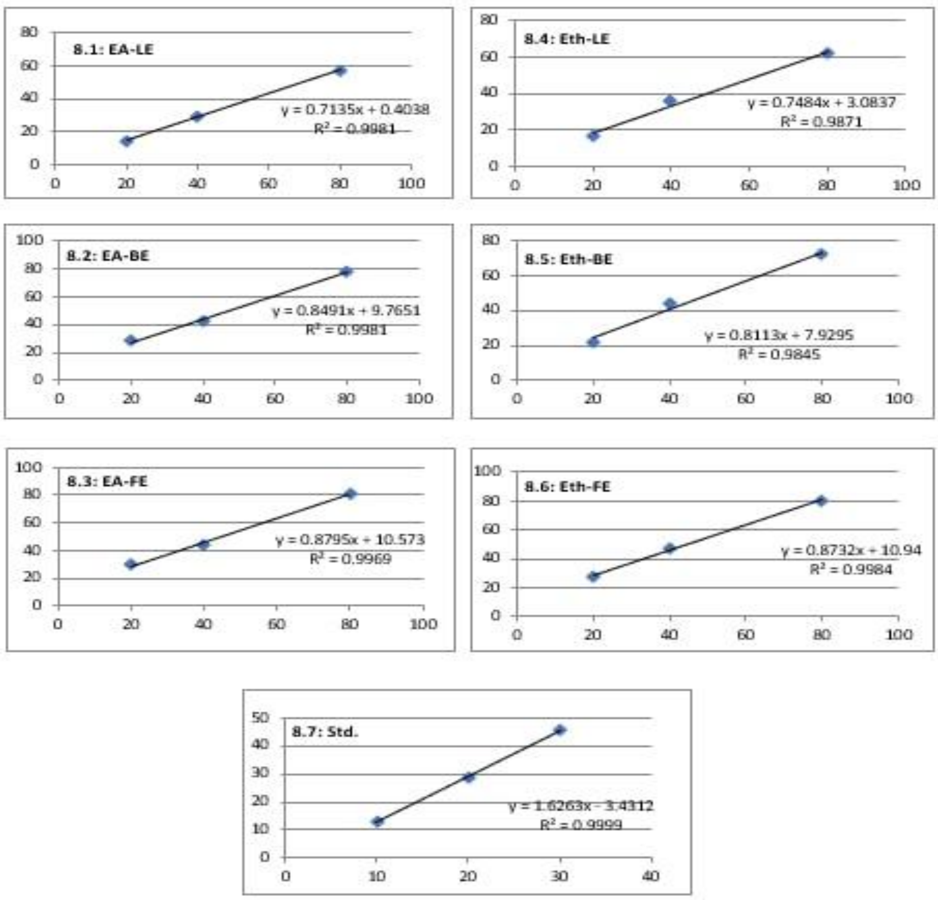


Figure 8.1-8.7 Represent the percent inhibition of haemolysis by different concentrations of extracts of *Firmiana colorata* compared to standard – L- ascorbic acid. Each value in the graph is represented as mean ± SD (n=3)

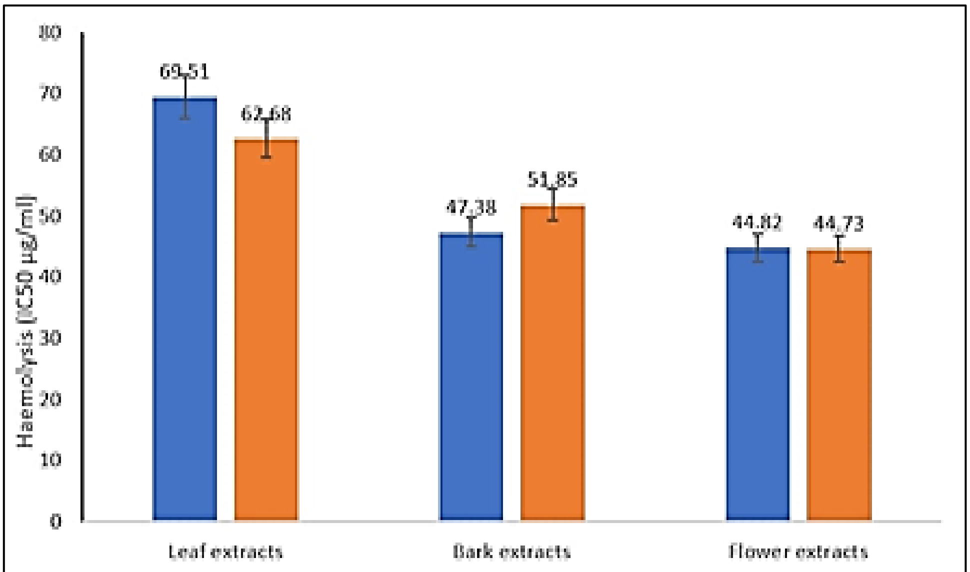


Figure 8.8: Antihaemolytic test effects of leaf, bark and flower extracts of *Firmiana colorata*. Each value in the graph is represented as mean ± SD (n=3)

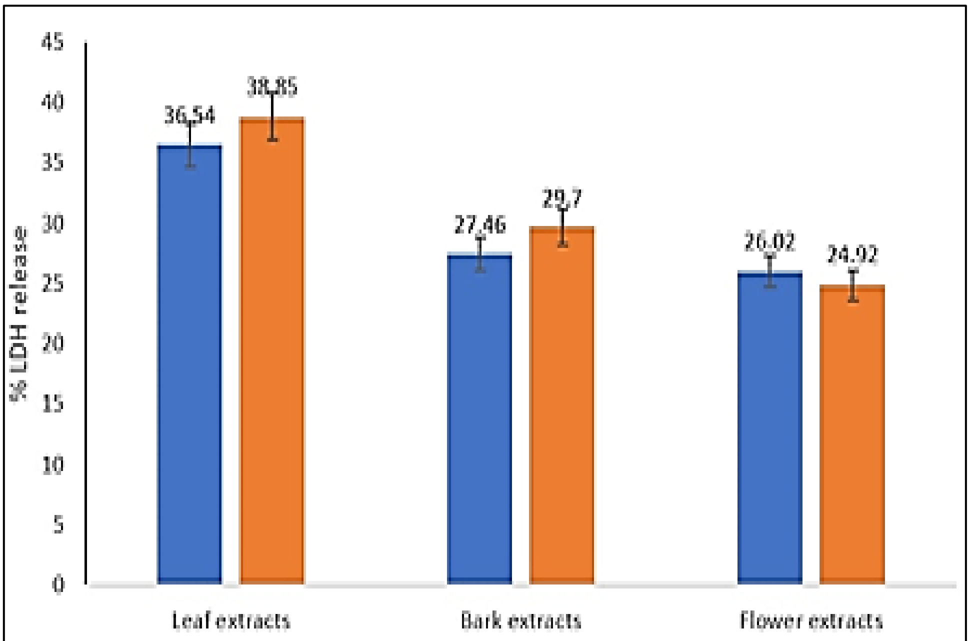


Figure 8.9: Lactate dehydrogenase test effects of leaf, bark and flower extracts of *Firmiana colorata*. Each value in the graph is represented as mean±SD (n=3)

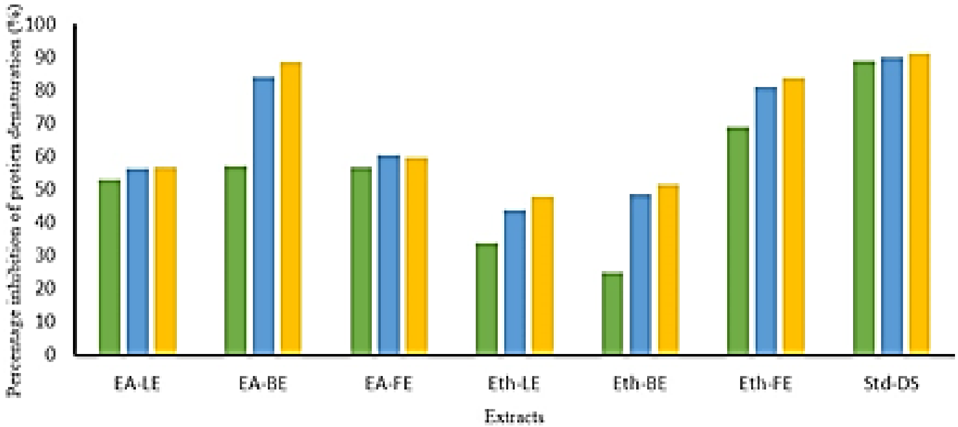


Figure 8.10: Percentage inhibition of protein denaturation exhibited by leaf, bark and flower extracts of *Firmiana colorata*. Each value in the graph is represented as mean ± SD (n=3)

at 200 µg/ml of extract   at 400 µg/ml of extract   at 800 µg/ml of extract

Conclusion

Firmiana colorata may be recommended as a plant of phytopharmaceutical importance as it contains several and a variety of bioactive compounds. The results of the current study can accelerate the development of a new class of plant-based anti-inflammatory agents to treat several inflammatory diseases.

Conflict of interest: Authors have declared that no competing interests exist.

Author Contributions:

All authors contributed to the conception and design of the study. Laboratory work, data collection and analysis were performed by Bi Bi Hajira. The manuscript was written and approved by Bi Bi Hajira and Geraldine Menezes.

References

1. Tyagi T, Agarwal M. Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of *Pistia stratiotes* L. and Eichhornia crassipes (Mart.) solms. Journal of Pharmacognosy and phytochemistry. 2017;6(1):195-206.
2. Keke CO, Nsofor WN, Kumabia FK, Iloabuchi GC, Ejiofor JC, Osuagwu OL. GCMS and FTIR analysis of ethanol and methanol leave extract of Urena lobata (Caesar weed) for bioactive phytochemical constituents. Journal of Drug Delivery and Therapeutics. 2023 Jan 15;13(1):99-115.
3. Belakhdar G, Benjouad A, Abdennebi EH. Determination of some bioactive chemical constituents from Thesium humile Vahl. J Mater Environ Sci. 2015;6(10):2778-83.
4. Krishnamoorthy K, Subramaniam P. Phytochemical profiling of leaf, stem, and tuber parts of Solena amplexicaulis (Lam.) Gandhi using GC-MS. International scholarly research notices. 2014;2014(1):567409.
5. Jabbar AA, Abdullah FO, Abdulrahman KK, Galali Y, Sardar AS. GC-MS Analysis of Bioactive Compounds in Methanolic Extracts of Papaver decaisnei and Determination of Its Antioxidants and Anticancer Activities. Journal of Food Quality. 2022;2022(1):1405157.
6. Gomathi D, Kalaiselvi M, Ravikumar G, Devaki K, Uma C. GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of Evolvulus alsinoides (L.) L. Journal of food science and technology. 2015 Feb;52:1212-7.
7. Konappa N, Udayashankar AC, Krishnamurthy S, Pradeep CK, Chowdappa S, Jogaiah S. GC–MS analysis of phytoconstituents from Amomum nilgiricum and molecular docking interactions of bioactive serverogenin acetate with target proteins. Scientific reports. 2020 Oct 2;10(1):16438.
8. Parvin MS, Das N, Jahan N, Akhter MA, Nahar L, Islam ME. Evaluation of *In vitro* anti-inflammatory and antibacterial potential of Crescentia cujete leaves and stem bark. BMC research notes. 2015 Dec;8:1-7.
9. Rodríguez-Yoldi MJ. Anti-inflammatory and antioxidant properties of plant extracts. Antioxidants. 2021 Jun 7;10(6):921.
10. Fagbemi KO, Aina DA, Olajuyigbe OO. Soxhlet extraction versus hydrodistillation using the clevenger apparatus: A comparative study on the extraction of a volatile compound from Tamarindus indica seeds. The Scientific World Journal. 2021;2021(1):5961586.
11. Bhattacharjee S, Ghosh C, Sen A, Lala M. Characterization of *Firmiana colorata* (Roxb.) R. Br. leaf extract and its silver nanoparticles reveal their antioxidative, anti-microbial, and anti-inflammatory properties. International Nano Letters. 2023 Dec;13(3):235-47.
12. Hajira B and Menezes G. Evaluation of Phytochemicals, mineral constituents and antimicrobial activity of leaf, bark and flower extracts of *Firmiana colorata*. *International journal of Pharma and Bio sciences.* 2021; *12*:1-11.
13. Pal RS, Kumar RA, Agrawal PK, Bhatt JC. Antioxidant capacity and related phytochemicals analysis of methanolic extract of two wild edible fruits from north western Indian Himalaya. Int J Pharm Bio Sci. 2013 Aug 28;4(2):113-23.
14. Zahoor M, Shafiq S, Ullah H, Sadiq A, Ullah F. Isolation of quercetin and mandelic acid from Aesculus indica fruit and their biological activities. BMC biochemistry. 2018 Dec;19:1-4.

15. Herborne J. Phytochemical methods. A guide to modern techniques of plant analysis. 2 5-11. DOI: 10.1007.
16. Bhuiyan MN, Begum J, Nandi NC, Akter F. Constituents of the essential oil from leaves and buds of clove (*Syzigium caryophyllatum* (L.) Alston). African Journal of Plant Science. 2010 Nov;4(11):451-4.
17. Lakshmegowda SB, Rajesh SK, Kandikattu HK, Nallamuthu I, Khanum F. *In vitro* and *in vivo* studies on hexane fraction of *Nitzschia palea*, a freshwater diatom for oxidative damage protective and anti-inflammatory response. Revista Brasileira de Farmacognosia. 2020 Apr;30:189-201.
18. Gulnaz AR, Kapfo W, Chauhan JB. *In vitro* anthelminthic and anti-inflammatory activity of ethanolic extract of *Randia uliginosa* DC Leaf. Int. J. Curr. Microbiol. App. Sci. 2014 Oct 15;3(9):793-9.
19. Sudarshan BL, Maheshwar PK, Priya PS, Sanjay KR. Volatile and phenolic compounds in freshwater diatom *Nitzschia palea* as a potential oxidative damage protective and anti-inflammatory source. Pharmacognosy Magazine. 2019, 15(64).
20. Naz R, Ayub H, Nawaz S, Islam ZU, Yasmin T, Bano A. Antimicrobial activity, toxicity and anti-inflammatory potential of methanolic extracts of four ethnomedicinal plant species from Punjab, Pakistan. BMC complementary and alternative medicine. 2017 Dec;17:1-3.
21. Ukwuani-Kwaja AN, Nwaogu J, Abba A. Antidiabetic, Antioxidant and Hypolipidemic Potentials of *Sterculia* Setigera Methanol Stem Bark Extract in Alloxan-Induced Diabetic Rats.
22. Silva FV, Oliveira IS, Figueiredo KA, Melo Junior FB, Costa DA, Chaves MH. Anti-inflammatory and antinociceptive effects of *Sterculia striata* a. St.-Hil. & Naudin (Malvaceae) in rodents. Journal of medicinal food. 2014 Jun 1;17(6):694-700.
23. Al Muqarrabun LM, Ahmat N. Medicinal uses, phytochemistry and pharmacology of family *Sterculia*ceae: A review. European journal of medicinal chemistry. 2015 Mar 6;92:514-30.
24. Daulatabad CD, Ankalgi RF. Cyclopropenoid fatty acids in *Sterculia colorata* seed oil. Journal of the American Oil Chemists Society. 1982 Oct;59(10):439.
25. Rabbi F, Nisar A. Two New Coumarins with Immunomodulatory and Anticancer Activities from *Sterculia colorata*. Chemistry of Natural Compounds. 2023 May;59(3):436-40.
26. Bhattacharjee S, Ghosh C, Sen A, Lala M. Characterization of *Firmiana colorata* (Roxb.) R. Br. leaf extract and its silver nanoparticles reveal their antioxidative, anti-microbial, and anti-inflammatory properties. International Nano Letters. 2023 Dec;13(3):235-47.
27. Al-Rahman AY. A pharmacognostical study of certain *Sterculia* species (Family: *Sterculia*ceae). Cairo University, Giza. 2017.
28. Prabha S, Kumar J. Gas Chromatographic and Mass Spectroscopic (GC-MS) Analysis of Rhizome of *Acorus calamus* Linn. for Identification of Potent Antimicrobial Bio-active Compounds. Journal of Scientific Research. 2021 Jan 1, 13(1).
29. Bhattacharjee S, Ghosh C, Sen A, Lala M. Characterization of *Firmiana colorata* (Roxb.) R. Br. leaf extract and its silver nanoparticles reveal their antioxidative, anti-microbial, and anti-inflammatory properties. International Nano Letters. 2023 Dec;13(3):235-47.
30. Wu CY, e Silva AC, Citadin CT, Clemons GA, Acosta CH, Knox BA. Palmitic acid methyl ester inhibits cardiac arrest-induced neuroinflammation and mitochondrial dysfunction. Prostaglandins, Leukotrienes and Essential Fatty Acids. 2021 Feb 1;165:102227.
31. Shin SA, Joo BJ, Lee JS, Ryu G, Han M, Kim WY. Phytochemicals as anti-inflammatory agents in animal models of prevalent inflammatory diseases. Molecules. 2020 Dec 15;25(24):5932.
32. Ambily PG, Mathew J, Sudhina M. Analysis of leaf extract of *Zingiber officinale* by a hybrid analytical technique. Current Trends in Biotechnology and Pharmacy. 2022 Aug 27;16(3):316-28.
33. Kulkarni A, Govindappa M, Chandrappa CP, Ramachandra YL, Koka PS. Phytochemical analysis of Cassia fistula and its *In vitro* antimicrobial, antioxidant and anti-inflammatory activities. Advancement in Medicinal Plant Research. 2015 Feb 9;3(1):8-17.
34. Bhardwaj M, Sali VK, Mani S, Vasanthi HR. Neophytadiene from *Turbinaria ornata* suppresses LPS-induced inflammatory response in RAW 264.7 macrophages and Sprague Dawley rats. Inflammation. 2020 Jun;43:937-50.
35. Saranya MS, Arunprasath A. Evaluation of phytochemical compounds in *Corbichonia decumbens* (Frossk). Excell by using Gas Chromatography-Mass Spectrometry. J. Appl. Adv. Res. 2019 Jun 7;4:89-93.
36. Nishanthini A, Mohan VR, Jeeva S. Phytochemical, ft-ir, and gc-ms analysis of stem and leaf of *Tiliacora acuminata* (lan.) Hook f & thomas (menispermaceae). International Journal of Pharmaceutical Sciences and Research. 2014 Sep 1;5(9):3977.
37. Igwe KK, Nwankwo PO, Otuokere IE, Ijioma SN, Amaku F. GCMS analysis of phytocomponents in the methanolic extract of Moringa oleifera leave. Journal of Research in Pharmaceutical science. 2015 Nov, 2(1).
38. Sivagurunathan A, Xavier BI. GC-MS evaluation of bioactive compounds of *Marsilea quadrifolia* Linn. Aquatic Fern. Int J Pharm Res Scholars. 2014;3(1):2277-7873.
39. Ganesh M, Mohankumar M. Extraction and identification of bioactive components in *Sida cordata* (Burm. f.) using gas chromatography–mass spectrometry. Journal of food science and technology. 2017 Sep;54:3082-91.
40. Achi NK, Ohaeri OC. GC-MS determination of bioactive constituents of the methanolic fractions of *Cnidoscolus aconitifolius*. British Journal of Pharmaceutical Research. 2015 Jan 1;5(3):163.
41. Achi NK, Ohaeri OC. GC-MS determination of bioactive constituents of the methanolic fractions of *Cnidoscolus aconitifolius*. British Journal of Pharmaceutical Research. 2015 Jan 1;5(3):163.
42. Sivagurunathan A, Xavier BI. GC-MS evaluation of bioactive compounds of *Marsilea quadrifolia* Linn. Aquatic Fern. Int J Pharm Res Scholars. 2014;3(1):2277-7873.
43. Ibrahim M, Ankwai GE, Taave P, Gungshik JR, Wapwera JA. Optimization of essential oil extraction from *Cymbopogon* spp (lemon grass) and its application

in perfume formulation. Journal of Chemical Society of Nigeria. 2021 Dec 31, 46(6).
44. Dulara BK, Godara P, Barwer N. *In-vivo* and *In-vitro* phytochemical GC-MS analysis of volatile constituents of *Andrographis paniculata* (Burm. f.) Nees. The Pharma Innovation Journal. 2019;8:255-61.
45. Marimuthu S, Karthic C, Mostafa AA, Al-Enazi NM, Abdel-Raouf N, Sholkamy EN. Antifungal activity of Streptomyces sp. SLR03 against tea fungal plant pathogen *Pestalotiopsis theae*. Journal of King Saud University-Science. 2020 Dec 1;32(8):3258-64.
46. Banakar P, Jayaraj M. GC-MS analysis of bioactive compounds from ethanolic leaf extract of *Waltheria indica* Linn. and their pharmacological activities. Int. J. Pharm. Sci. Res. 2018 May 1;9(5):2005-10.
47. De Moraes J, de Oliveira RN, Costa JP, Junior AL, de Sousa DP, Freitas RM, Pinto PL. Phytol, a diterpene alcohol from chlorophyll, as a drug against neglected tropical disease *Schistosomiasis mansoni*. PLOS Neglected Tropical Diseases, 2014; 8(1).
48. Hadi I, Hussein HM. Antimicrobial Activity and spectral chemical analysis of methanolic leaves extract of Adiantum Capillus-Veneris using GC-MS and FT-IR spectroscopy. Int J Pharmacogn Phytochem Res. 2016;8(3):369-85.
49. Sulthanabegam M, Palani S, Senthikumar B. Phytoconstituents screening by GC-MS and evaluation of *In-vitro* Gastroprotective and antioxidant activity of Vulva lactuca. Int J Pharm Chem Biol Sci. 2019;9(2):939-50.
50. Prabha S, Kumar J. Gas Chromatographic and Mass Spectroscopic (GC-MS) Analysis of Rhizome of *Acorus calamus* Linn. for Identification of Potent Antimicrobial Bio-active Compounds. Journal of Scientific Research. 2021 Jan 1, 13(1).
51. Chan HH, Sun HD, Reddy MV, Wu TS. Potent α-glucosidase inhibitors from the roots of *Panax japonicus* CA Meyer var. major. Phytochemistry. 2010 Aug 1;71(11-12):1360-4.
52. Chan HH, Sun HD, Reddy MV, Wu TS. Potent α-glucosidase inhibitors from the roots of *Panax japonicus* CA Meyer var. major. Phytochemistry. 2010 Aug 1;71(11-12):1360-4.
53. Banakar P, Jayaraj M. GC-MS analysis of bioactive compounds from ethanolic leaf extract of *Waltheria indica* Linn. and their pharmacological activities. Int. J. Pharm. Sci. Res. 2018 May 1;9(5):2005-10.
54. Murugesan Amudha MA, Shanmugam Rani SR. GC-MS analysis of bioactive components of *Cordia retusa* (Boraginaceae).
55. Sayed-Ahmad B, Hijazi A, Bazzi H, Rammal H, Bazzal AE, Annan H. Determination of bioactive molecules and antioxidant activity in stinging nettle (*Urtica dioica*). J Multidisc Eng Sci Technol. 2015;2(10):2753-8.
56. Sayed-Ahmad B, Hijazi A, Bazzi H, Rammal H, Bazzal AE, Annan H. Determination of bioactive molecules and antioxidant activity in stinging nettle (*Urtica dioica*). J Multidisc Eng Sci Technol. 2015;2(10):2753-8.
57. Khan IH, Javaid A, Ahmed D, Khan U. Identification of volatile constituents of ethyl acetate fraction of *Chenopodium quinoa* roots extract by GC-MS. Int. J. Biol. Biotechnol. 2020 Jun 4;17(1):17-21.