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ATR-FTIR and GC-MS Analysis of Potentially Bioactive Compounds From *Bridelia ferruginea* Bark, *Khaya senegalensis* Bark and *Psidium guajava* Leaves

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Abstract
Plants are of therapeutic value due to their diverse phytochemicals that confer numerous pharmacological benefits. Bridelia ferruginea, Khaya senegalensis, and Psidium guajava, contain bioactive components with potential therapeutic applications, particularly in treating gastrointestinal diseases. To investigate their chemical composition, the bark of Brideliaferruginea, Khaya senegalensis bark, and Psidium guajava leaves were analyzed using Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR), and Gas Chromatography-Mass Spectrometry (GC-MS). The functional groups and chemical compositions were identified and quantified using advanced spectrometers from Agilent Technologies. The GC-MS analysis revealed distinct phytochemical profiles for each plant, with 18 compounds detected in B. ferruginea, 11 in K. senegalensis, and 32 in P. guajava. Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) analysis confirmed the presence of phenols, alcohols, aromatic compounds, esters, ethers in all three plants. These findings suggest that the bioactive compounds in these plant extracts may contribute to their pharmacological activities, particularly in the treatment of gastrointestinal diseases.

Keywords: *Bridelia ferruginea, Khaya senegalensis, Psidium guajava,* Bioactivity, Functional groups

INTRODUCTION
The application of medicinal plants against gastrointestinal diseases has attracted growing interest recently due to their therapeutic potentials and the increasing awareness towards alternative medicine. Of these plants, *Bridelia ferruginea, Khaya senegalensis* and *Psidium guajava* are the most popularly used in traditional medicine (Sulaiman *et al.,* 2022).These plants are important in the health sector among locals, traditional medical practitioners and they also have diverse bioactive compounds which make them medicinal plants. *Bridelia ferruginea*, (Euphorbiaceae), is known to possess antioxidant activity attributable to its diverse phytochemical composition, encompassing flavonoids and alkaloids. Studies have shown that extracts derived from this plant possess inhibitory effects on lipid peroxidation, thereby mitigating oxidative stress-induced tissue damage (Adeleye, 2020).The antioxidant properties

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of *Bridelia ferruginea* hold significant implications for gastrointestinal health, as oxidative stress exacerbates various digestive disorders (Gholamnezhad *et al.,* 2018). Moreover, traditional medicinal applications of this plant, particularly in the management of dysentery and diarrhoea, underscores its ethnopharmacological relevance (Alowanou *et al.,* 2015).

Khaya senegalensis, commonly known as African mahogany, has diverse medicinal properties. Traditionally, this plant has been employed to combat gastrointestinal disorders, specifically alleviating symptoms of diarrhoea and dysentery (Senouci *et al.,* 2022). The therapeutic effects of *Khaya senegalensis* can be attributed to its potent anti-inflammatory and antimicrobial activities, which effectively manage infections affecting the gastrointestinal tract (Karimi *et al.,* 2017).Numerous ethnobotanical studies revealed the plant's efficacy in treating digestive issues, highlighting its importance in traditional healing practices (Senouci *et al.,* 2022; Es-safi *et al.,* 2020). These findings demonstrate the potential of *Khaya senegalensis* as a valuable natural remedy for gastrointestinal health.

Psidium guajava, commonly known as guava, is a medicinal plant with a long history of treating gastrointestinal issues. The leaves of this versatile plant have been traditionally used to alleviate diarrhoea, dysentery, and other digestive disorders, thanks to their astringent properties and ability to regulate intestinal motility (Bahmani *et al.,* 2014). Previous studies have validated its therapeutic potential, revealing that its leaf extracts possess potent antimicrobial properties effective against pathogens responsible for gastrointestinal infections (Palombo, 2006). The widespread cultural use of *Psidium guajava* for digestive health underscores its significance as a natural remedy, with diverse traditional practices attesting to its efficacy (Pérez-Ochoa *et al.,* 2019).

This study has the potential to uncover detailed chemical profiles of these plants that are currently underexplored, and bridges the gap between phytochemical analysis and pharmacological application, leading to a better understanding of the mechanisms underlying the medicinal properties of these plants

METHODOLOGY

Plant Collection and Identification

The plant species used in this study were selected based on a comprehensive literature review, field surveillance, and a questionnaire administered to traditional medical practitioners in Kano State, Nigeria. A taxonomist at the herbarium unit of Bayero University Kano, Department of Biological Sciences identified the plants. Three species – *Bridelia ferruginea*, *Khaya senegalensis*, and *Psidium guajava* - were identified and assigned voucher numbers BUKHAN 0049, BUKHAN 0116, and BUKHAN 0336, respectively.

Pre-treatment of Plant Parts

The leaves were gently wiped clean with a cloth and then laid out alongside the stem bark in the laboratory to air-dry at ambient temperature. Once thoroughly dried, the plant materials were ground into a fine powder using a mortar and pestle. The resulting powders were stored in clean, screw-cap containers and labelled for future reference.

Extraction of Plant

The extraction protocol employed was based on the methodology described by Sulaiman *et al.* (2024). Briefly, 200 g of the pulverized plant material was loaded into a Soxhlet thimble and subjected to solvent extraction using 1 L of 95% (v/v) ethanol. The extraction process was

continued until exhaustion, as indicated by the absence of colour change in the ethanol solvent. The resulting ethanolic extract was then concentrated under reduced pressure using a rotary evaporator, yielding a crude extract. Residual solvent was removed by drying the extract in porcelain dishes at 80°C until a constant weight was achieved. The dried extract was then transferred to a clean, screw-cap container, labelled, and stored at 4°C for future use.

Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) Spectroscopy Exactly 10 mg of each crude extract was placed on the diamond crystal of the ATR accessory and flattened using a compression clamp to ensure optimal contact. ATR-FTIR spectra were recorded using a Cary 630 spectrometer (Agilent Technologies, USA) over the range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1} and 32scans (Fadare *et al.,* 2015).

Gas Chromatography- Mass Spectrometry (GC-MS) Analysis
Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed on the ethanolic extracts of *Khaya senegalensis* bark, *Bridelia ferruginea* bark, and *Psidium guajava* leaves using an Agilent Technologies GC-7890B/MSD-5977A system, equipped with an HP-5MS UI column (30m x 250µm x 0.25µm). The GC-MS detection employed electron ionization at 70eV, with high-purity Helium (99.995%) as the carrier gas at a flow rate of 1mL/min. The temperature program consisted of an initial gradient from 50°C to 150°C at 3°C/min, followed by a 10-minute isothermal hold, and a final ramp to 300°C at 10°C/min. One microliter of each 1% extract, diluted in an appropriate solvent, was injected in split less mode, and the relative abundance of each chemical component was quantified based on chromatogram peak areas which were interpreted using the National Institute of Standards and Technology (NIST) database, which contains over 62,000 patterns, to identify known compounds by matching their spectra with the NIST library (Buss and Butler, 2010).

RESULTS
ATR-FTIR spectrometer is a powerful instrument that can be used to determine the functional groups of compounds and chemical bonds of specific molecules. This accessory provides for the non-destructive infrared measurement of both transmittance and absorbance spectra of samples with little or no preparation. Figure 1-3 shows the ATR-FTIR absorption spectra of ethanolic extracts of *Bridelia ferruginea* bark, *Khaya senegalensis* bark and *Psidium guajava* leaves respectively. The peak values and its corresponding functional group for each extract is indicated in Tables 1-3 respectively.

Table 1: Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) Profile of Ethanolic Extract of *Brideliaferruginea* bark

Peak Value (cm^{-1})	Functional Group
3198.1	OH Stretching (Hydroxyl Group)
2105.9	C≡N Stretching (Nitrile Group)
1908.4	C=C Stretching (Aromatic Ring)
1602.8	C=C Stretching (Aromatic Ring)
1520.8	C=C Stretching (Aromatic Ring)

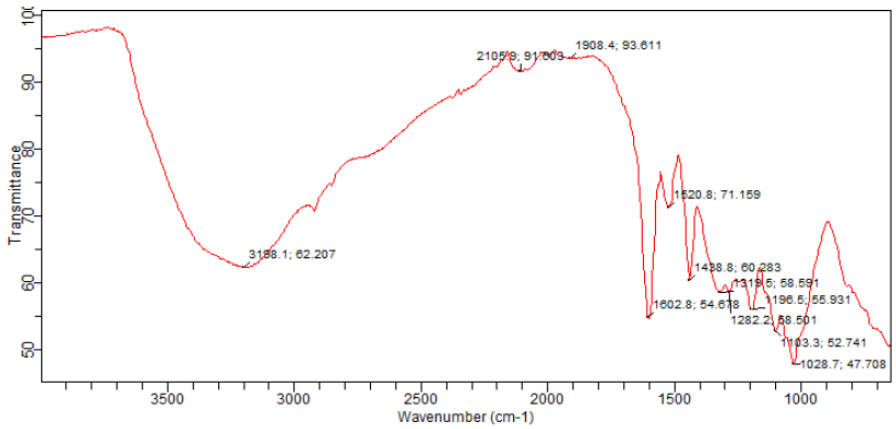


Figure 1: Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) Spectrum of Ethanolic Extract of *Bridelia ferruginea* bark

Table 2: Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) Profile of Ethanolic Extract of *Khaya senegalensis* bark

Peak Value (cm^{-1})	Functional Group
3209.2	OH Stretching (Alcoholic or Phenolic,) - likely a Hydroxyl (OH) group
2922.2	C-H Stretching (Alkane or Alkyl Group) - likely a Methyl (CH3) or Methylene (CH2) group
2120.9	C≡N Stretching (Nitrile Group) or C≡C Stretching (Alkyne Group) - likely a Nitrile (CN) group
1871.1	C=O Stretching (Unsubstituted Aldehyde or Ketone) or C=C Stretching (Aromatic Ring) - likely an Unsubstituted Aldehyde (CHO) or Ketone (CO) group
1699.7	C=O Stretching(Carboxylic Acid or Esters)
1602.8	C=C Stretching (Aromatic Ring)
1520.8	C=C Stretching (Aromatic Ring)

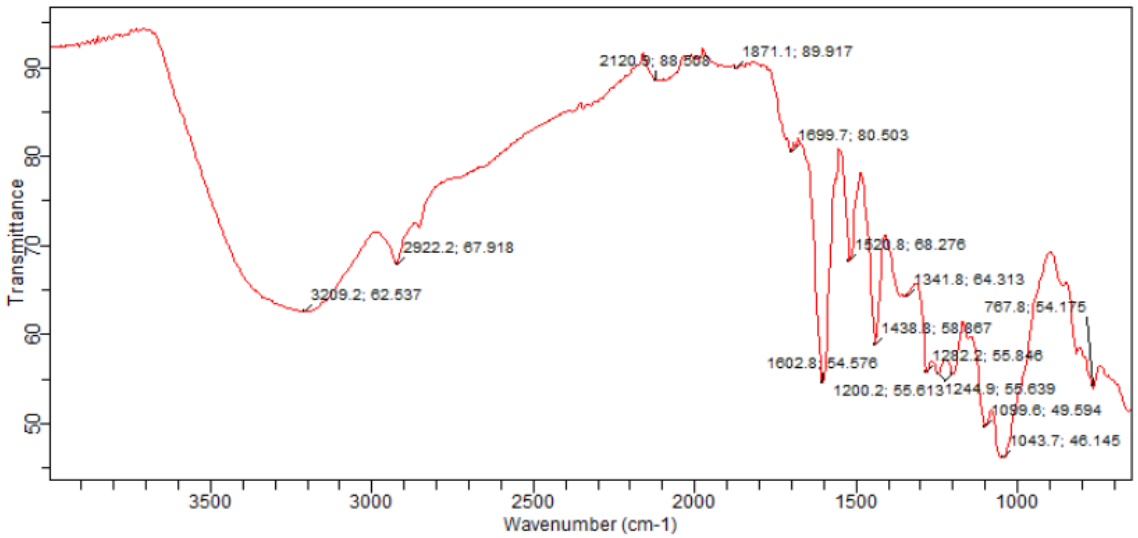


Figure 2: Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) Spectrum of Ethanolic Extract of *Khaya senegalensis* bark

Table 3: Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) Profile of Ethanolic Extract of *Psidium guajava* leaves

Peak Value (cm^{-1})	Functional Group
3235.3	OH Stretching (Alcoholic, Phenolic, or Hydroxyl Group) - likely a Hydroxyl (OH) group
2918.5	C-H Stretching (Alkane or Alkyl Group) - likely a Methyl (CH3) or Methylene (CH2) group
2851.4	C-H Stretching (Alkane or Alkyl Group) - likely a Methyl (CH3) or Methylene (CH2) group
2113.4	C≡N Stretching (Nitrile Group) or C≡C Stretching (Alkyne Group) - likely a Nitrile (CN) group
1684.8	C=O Stretching(Carboxylic Acid or Esters)
1606.5	C=C Stretching (Aromatic Ring)
1520.8	C=C Stretching (Aromatic Ring)

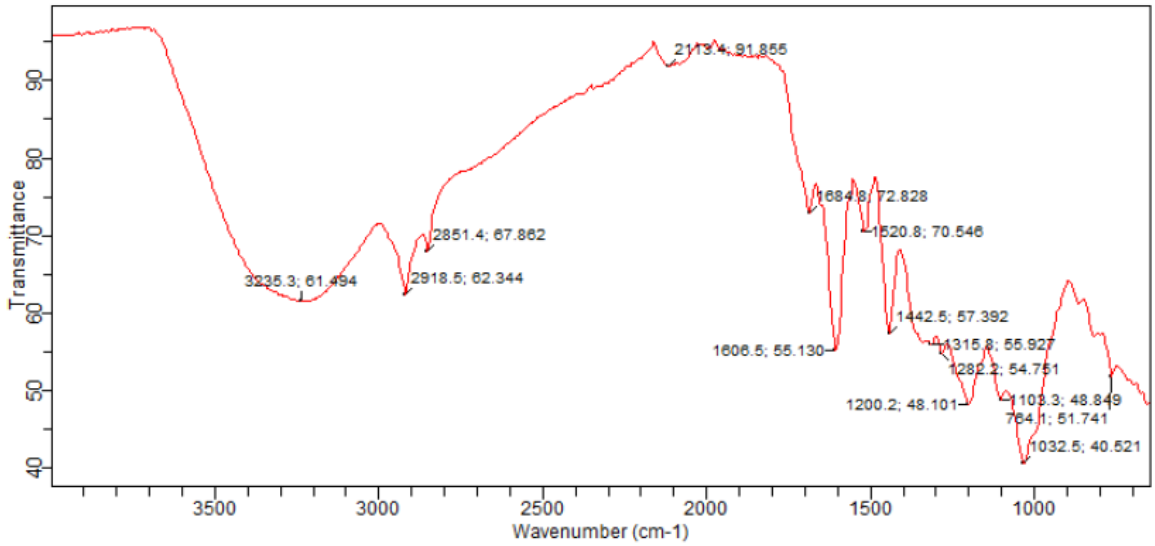


Figure 3: (FT-IR) Spectrum of Ethanolic Extract of *Psidium guajava* Leaves

Gas chromatography-mass spectrometry (GC-MS) analysis was performed on ethanolic extracts of *Brideliaferruginea* bark, *Khaya senegalensis* bark, and *Psidium guajava* leaves. The resulting chromatograms (Figures 7-9) exhibited distinct peak profiles, indicative of diverse phytochemical constituents. Quantification of peak areas revealed varying concentrations of detected compounds. Subsequent identification and characterization of the compounds yielded 18 bioactive molecules in *B. ferruginea*, 11 in *K. senegalensis*, and 32 in *P. guajava* (Tables 7-9). The compiled data encompass retention times, peak areas, compound names, molecular formulas, and molecular weights, providing valuable insights into the phytochemical composition of these plant extracts

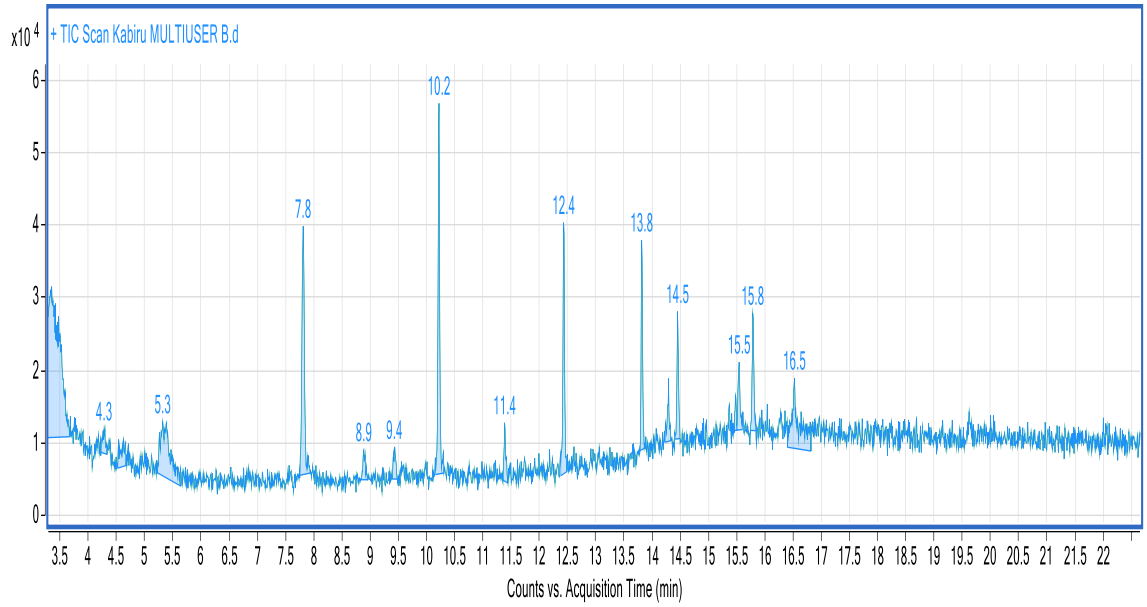
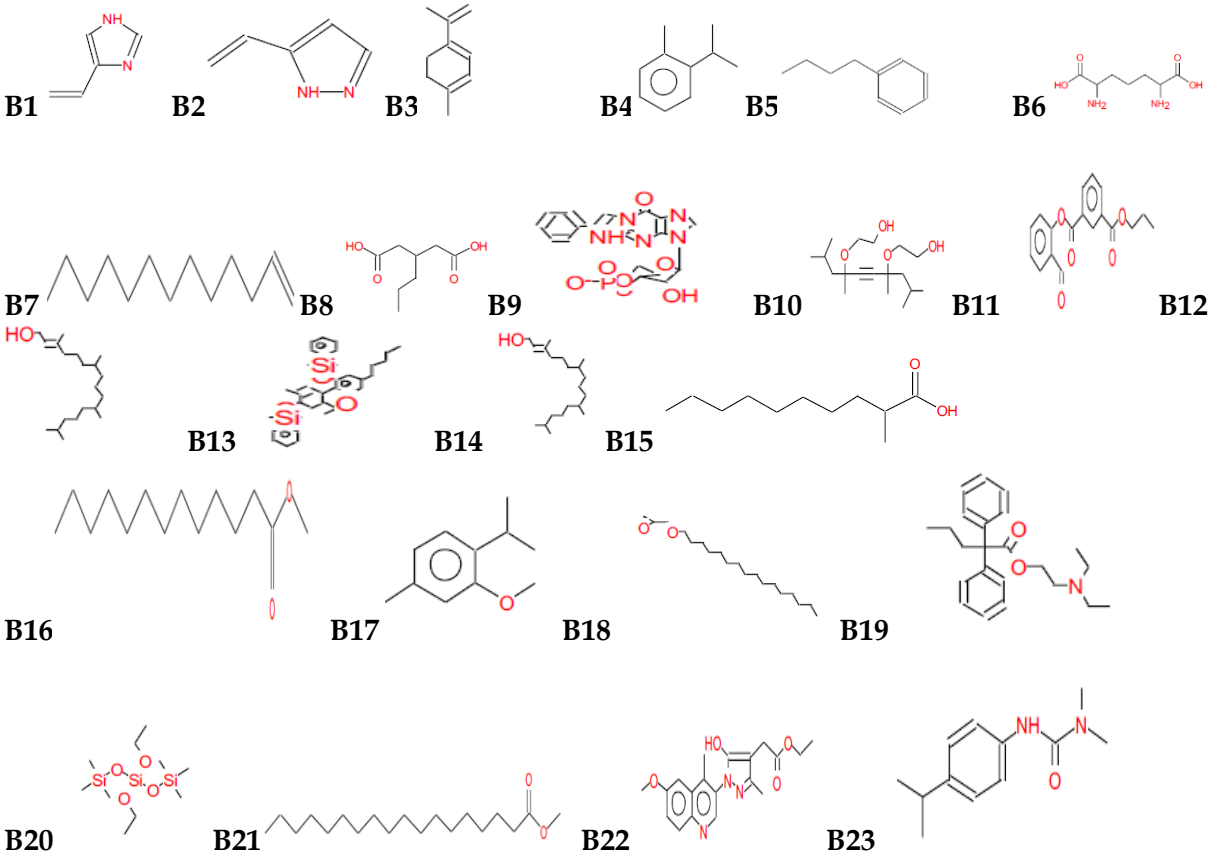


Figure 4: GC-MS Chromatograph of Ethanolic extract of *Bridelia ferruginea* bark

Table 4: Bioactive compounds found in Ethanolic Extract of *Bridelia ferruginea* bark using GC-MS

Peak	RT	Area	Name of Compound	Molecular formula	Molecular Structure	Molecular weight
1	3.4	367851.21	4-Vinyl-imidazole 5-Vinyl-pyrazole	$C_5H_6N_2$ $C_5H_6N_2$	B1 B2	94 94
2	4.3	12115.83	1,3,8-p-Menthatriene	$C_{10}H_{14}$	B3	134
3	4.6	21030.09	o-Cymene n-Butylbenzene	$C_{10}H_{14}$ $C_{10}H_{14}$	B4 B5	134 134
4	5.3	93891.5	2,6-Diaminopimelic acid	$C_7H_{14}N_2O_4$	B6	190
5	7.8	108033.82	1-Tridecene	$C_{13}H_{26}$	B7	182
6	8.9	12225.89	3-Propylglutaric acid PET-cGMP	$C_8H_{14}O_4$ $C_{18}H_{15}N_5O_7P$	B8 B9	174 444
7	9.4	12883.46	Decynediol, tetramethyl, di(2-hydroxyethyl) ether Isophthalic acid, 2-formylphenyl propyl ester	$C_{18}H_{34}O_4$ $C_{18}H_{16}O_5$	B10 B11	314 312
8	10.2	108833.29	Phytol	$C_{20}H_{40}O$	B12	296
9	11.4	17852.61	Silane, [(6a,7,8,10a-tetrahydro-6,6,9-trimethyl-3-pentyl-6H-dibenzo[b,d]pyran -1,8-diyl)bis(oxy)]bis[dimethylphenyl-, [6ar-(6aα,8β,10aβ)]-	$C_{37}H_{50}O_3Si_2$	B13	598
10	12.4	73711.94	Phytol	$C_{20}H_{40}O$	B14	296

11	13.8	51751.32	Decanoic acid, 2-methyl- Tridecanoic acid, methyl ester	$C_{11}H_{22}O_2$ $C_{14}H_{28}O_2$	B15 B16	186 228
12	14.3	18443.54	Benzene, 2-methoxy-4-methyl-1-(1-methylethyl)-	$C_{11}H_{16}O$	B17	164
13	14.5	32893.59	Oxirane, [(hexadecyloxy)methyl]-	$C_{19}H_{38}O_2$	B18	298
14	15.5	7316.7	Proadifen	$C_{23}H_{31}NO_2$	B19	353
15	15.5	23549.44	Silicic acid, diethyl bis(trimethylsilyl) ester	$C_{10}H_{28}O_4Si_3$	B20	296
16	15.8	3438.36	Methyl stearate	$C_{19}H_{38}O_2$	B21	298
17	16.5	87501.4	Ethyl [5-hydroxy-1-(6-methoxy-4-methyl-3-quinolinyl)-3-methyl-1H-pyrazol-4-yl]acetate	$C_{19}H_{21}N_3O_4$	B22	355
18	23.5	94288.73	Isoproturon Genistin 8-Isopropyl-5-methyl-5,6,7,8-tetrahydro-2,4-quinazolinedione	$C_{12}H_{18}N_2O$ $C_{21}H_{20}O_{10}$ $C_{12}H_{18}N_2O_2$	B23 B24 B25	206 432 222



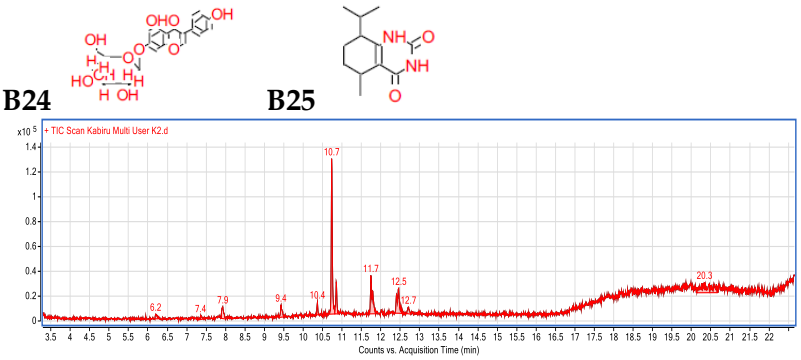
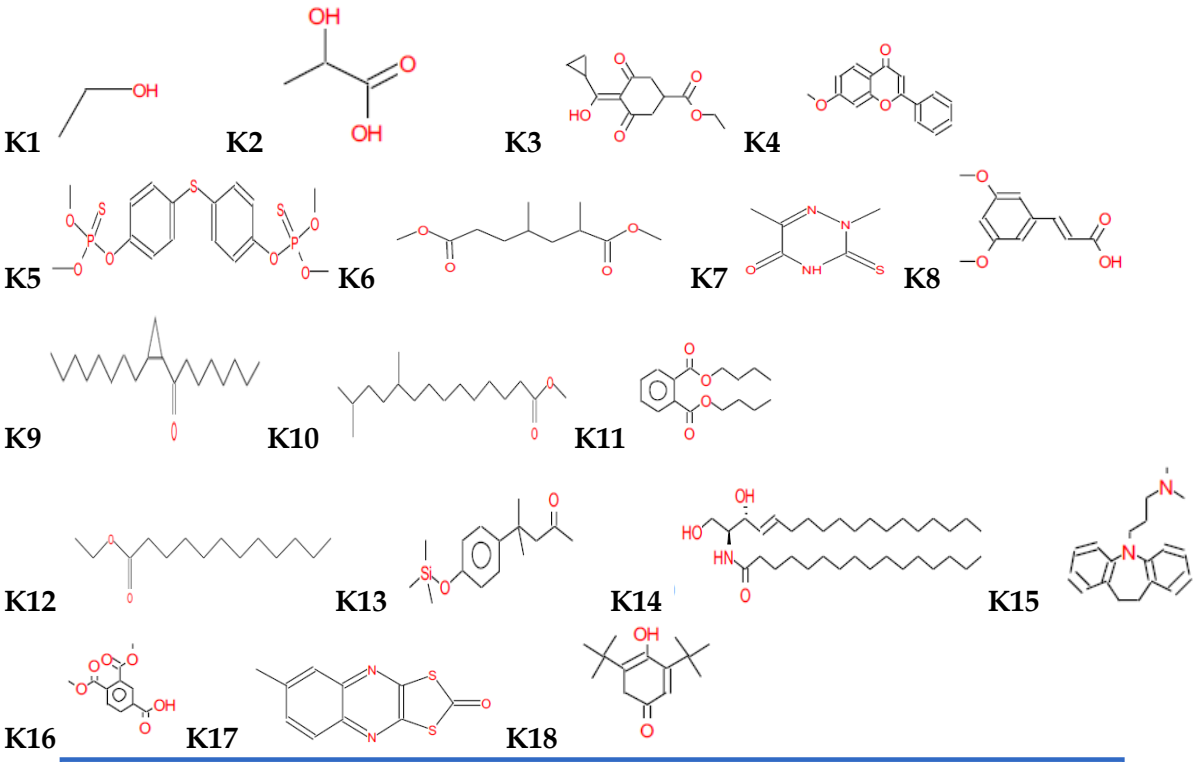


Figure 5: GC-MS Chromatograph of Ethanolic extract of *Khaya senegalensis* leaves

Table 5: Bioactive compounds found in Ethanolic Extract of *Khaya senegalensis* bark using GC-MS

Peak	RT	Area	Name of Compound	Molecular formula	Molecular Structure	Molecular weight
1	6.2	13194.66	Ethanol	C_2H_6O	K1	46
			L-Lactic acid	$C_3H_6O_3$	K2	90
2	7.4	10907.47	Trinexapac-ethyl	$C_{13}H_{16}O_5$	K3	252
			7-Methoxyflavanone	$C_{16}H_{12}O_3$	K4	252
			Temefos	$C_{16}H_{20}O_6P_2S_3$	K5	466
3	7.9	34763.3	(2S,4S)-2,4-Dimethylheptanedioic acid dimethyl ester	$C_{11}H_{20}O_4$	K6	216
			2,6-Dimethyl-3-thioxo-5-oxo-2,3,4,5-tetrahydro-1,2,4-triazine	$C_5H_7N_3OS$	K7	157
4	9.4	28853.99	3,5-Dimethoxycinnamic acid	$C_{11}H_{12}O_4$	K8	208
			1-Octanone, 1-(2-octylcyclopropyl)-	$C_{19}H_{36}O$	K9	280
5	10.4	16330.07	Tetradecanoic acid, 10,13-dimethyl-, methyl ester	$C_{17}H_{34}O_2$	K10	270
6	10.7	256925.24	Dibutyl phthalate	$C_{16}H_{22}O_4$	K11	278
7	10.9	55315.7	Dodecanoic acid, ethyl ester	$C_{14}H_{28}O_2$	K12	228
8	11.7	111464.18	Trimethyl[4-(2-methyl-4-oxo-2-pentyl)phenoxy]silane	$C_{15}H_{24}O_2Si$	K13	264
9	12.5	93660.37	Palmitoyl ceramide	$C_{34}H_{67}NO_3$	K14	537
			Imipramine	$C_{19}H_{24}N_2$	K15	280
10	12.7	18396.55	1,2,4-Benzenetricarboxylic acid, 1,2-dimethyl ester	$C_{11}H_{10}O_6$	K16	238
			Quinomethionate	$C_{10}H_6N_2OS_2$	K17	234
11	20.3	136620.59	2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-	$C_{14}H_{22}O_2$	K18	222



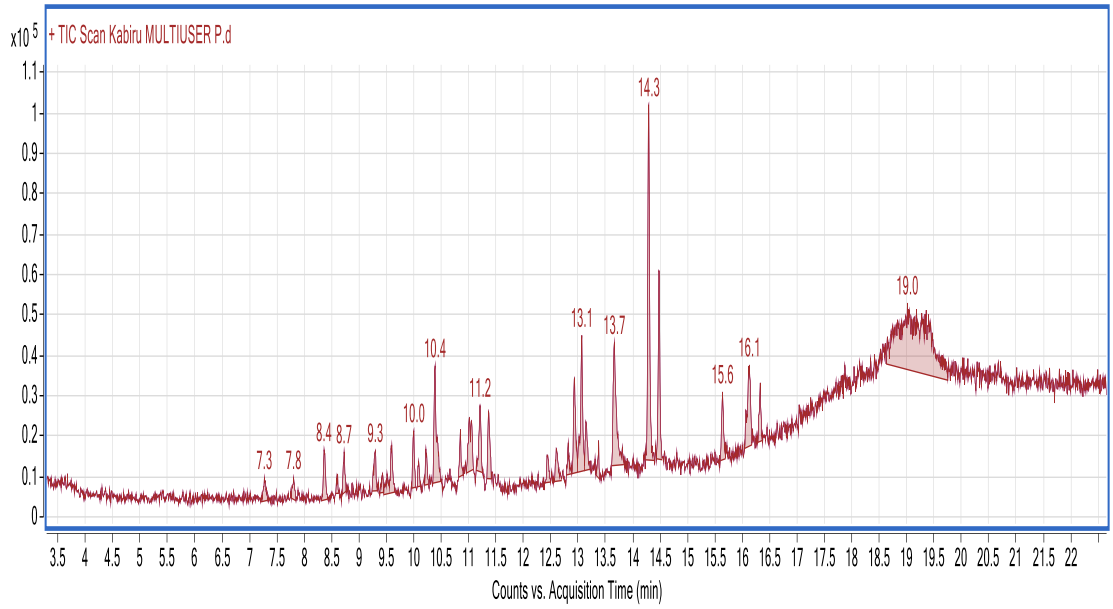


Figure 6: GC-MS Chromatograph of Ethanolic Extract of *Psidium guajava* Leaves

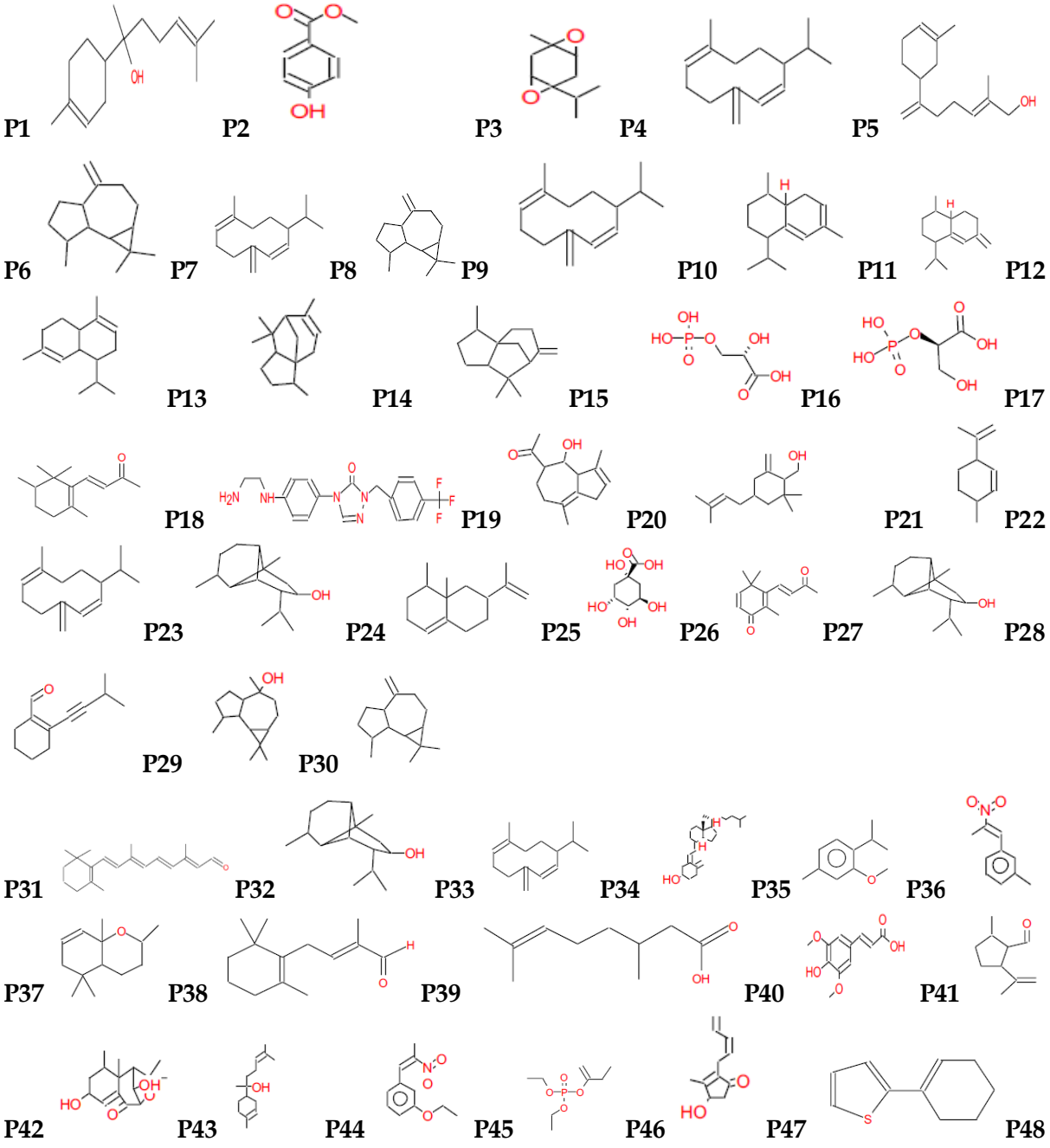
Table 6: Bioactive compounds found in Ethanolic Extract of *Psidium guajava* Leaves using GC-MS

Peak	RT	Area	Name of Compound	Molecular formula	Molecular Structure	Molecular weight
1	7.3	19989.91	α-Bisabolol	$C_{15}H_{26}O$	P1	222
			Methyl 4-hydroxybenzoate	$C_8H_8O_3$	P2	152
2	7.8	15146.27	Dioxatricyclo[5.1.0.0(3,5)]octane, 1-methyl-5-(1-methylethyl)-, (1α,3α,5α, 7α)-	$C_{10}H_{16}O_2$	P3	168
			Germacrene D	$C_{15}H_{24}$	P4	204

3	8.4	34403.72	6-(3-Methyl-3-cyclohexenyl)-2-methyl-2,6-heptadienol	$C_{15}H_{24}O$	P5	220
			Aromandendrene	$C_{15}H_{24}$	P6	204
4	8.6	7011.81	Germacrene D	$C_{15}H_{24}$	P7	204
			Aromandendrene	$C_{15}H_{24}$	P8	204
5	8.7	22235.26	Germacrene D	$C_{15}H_{24}$	P9	204
			cis-muurola-3,5-diene	$C_{15}H_{24}$	P10	204
			cis-muurola-4(14),5-diene	$C_{15}H_{24}$	P11	204
6	9.3	30369.28	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	$C_{15}H_{24}$	P12	204
			1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3α,3aβ, 7β,8aα)]-	$C_{15}H_{24}$	P13	204
			Cedrene	$C_{15}H_{24}$	P14	204
7	9.4	11120.06	3-Phosphoglyceric acid	$C_3H_7O_7P$	P15	186
			2-Phosphoglyceric acid	$C_3H_7O_7P$	P16	186
			3-Buten-2-one, 4-(2,5,6,6-tetramethyl-1-cyclohexen-1-yl)-	$C_{14}H_{22}O$	P17	206
8	9.6	49098.43	3H-1,2,4-Triazol-3-one, 4-[4-[(2-aminoethyl)amino]phenyl]-2,4-dihydro -2-[[4-(trifluoromethyl)phenyl]methyl]-	$C_{18}H_{18}F_3N_5O$	P18	377
			Ethanone, 1-(1,3a,4,5,6,7-hexahydro-4-hydroxy-3,8-dimethyl-5-azulenyl)-	$C_{14}H_{20}O_2$	P19	220
9	10	30794.6	1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane	$C_{15}H_{26}O$	P20	222
			Cyclohexene, 3-methyl-6-(1-methylethenyl)-, (3R-trans)-	$C_{10}H_{16}$	P21	136
10	10.1	11702.07	Germacrene D	$C_{15}H_{24}$	P22	204
			Dihydro-cis-α-copaene-8-ol	$C_{15}H_{26}O$	P23	222
			Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1α, 7β,8aα)]-	$C_{15}H_{24}$	P24	204
11	10.2	19261.38	Quinic acid	$C_7H_{12}O_6$	P25	192
			2,3-Dehydro-4-oxo-β-ionone	$C_{13}H_{16}O_2$	P26	204

12	10.4	94417.1	Dihydro-cis-α-copaene-8-ol	$C_{15}H_{26}O$	P27	222
			2-(3-Methyl-but-1-ynyl)-cyclohexene-1-carboxaldehyde	$C_{12}H_{16}O$	P28	176
			Viridiflorol	$C_{15}H_{26}O$	P29	222
13	10.9	21918.88	Aromandendrene	$C_{15}H_{24}$	P30	204
			all trans-Retinal	$C_{20}H_{28}O$	P31	284
14	11	60751.03	Dihydro-cis-α-copaene-8-ol	$C_{15}H_{26}O$	P32	222
15	11.2	45843.47	Germacrene D	$C_{15}H_{24}$	P33	204
16	11.4	2791.26	Cholecalciferol	$C_{27}H_{44}O$	P34	384
17	12.4	18360	Benzene, 2-methoxy-4-methyl-1-(1-methylethyl)-	$C_{11}H_{16}O$	P35	164
			cis-3-Methyl-β-methyl-β-nitrostyrene	$C_{10}H_{11}NO_2$	P36	177
18	12.6	27640.01	2H-1-Benzopyran, 3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl-, (2α,4aα,8aα)-	$C_{13}H_{22}O$	P37	194
			2-Butenal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	$C_{14}H_{22}O$	P38	206
19	12.8	16529.81	Citronellic acid	$C_{10}H_{18}O_2$	P39	170
			Sinapic acid	$C_{11}H_{12}O_5$	P40	224
20	12.9	66030.83	photocitral A	$C_{10}H_{16}O$	P41	152
21	13.1	96072.94	1,4-Methano-3-benzoxepin-5(4H)-one, 1,2,7,8,9,9a-hexahydro-7,10-dihydroxy-2,2,9,9a-tetramethyl-	$C_{15}H_{22}O_4$	P42	266
22	13.1	34133.83	Cyclohexene-1-methanol, α,4-dimethyl-α-(4-methyl-3-pentenyl)-	$C_{15}H_{26}O$	P43	222
			trans-3-Ethoxy-b-methyl-b-nitrostyrene	$C_{11}H_{13}NO_3$	P44	207
			Phosphoric acid, diethyl 1-methylenepropyl ester	$C_8H_{17}O_4P$	P45	208
23	13.4	14374	2-Cyclopenten-1-one, 4-hydroxy-3-methyl-2-(2,4-pentadienyl)-	$C_{11}H_{14}O_2$	P46	178
24	13.7	127419.2	1-Thienylcyclohexene	$C_{10}H_{12}S$	P47	164
			Calarene epoxide	$C_{15}H_{24}O$	P48	220
25	14.3	214562.6	Dibutyl phthalate	$C_{16}H_{22}O_4$	P49	278
26	14.5	101748.28	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	P50	284
27	15.6	42385.92	1-Naphthalenepentanoic acid, decahydro-2-hydroxy-β,2,5,5,8a-pentamethyl-6 -oxo-, methyl ester	$C_{21}H_{36}O_4$	P51	352
			Decahydro-8a-ethyl-1,1,4a,6-tetramethylnaphthalene	$C_{16}H_{30}$	P52	222

28	16.1	83148.43	Trimethyl[4-(2-methyl-4-oxo-2-pentyl)phenoxy]silane	$C_{15}H_{24}O_2Si$	P53	264
29	16.3	30945.52	4H-1-Benzopyran-4-one, 6,7-dimethoxy-3-phenyl-	$C_{17}H_{14}O_4$	P54	282
30	19	604080.23	No similar spectra in NIST Library			
31	25.5	64087.33	Silicic acid, diethyl bis(trimethylsilyl) ester	$C_{10}H_{28}O_4Si_3$	P55	296
32	26.3	30747.61	Sterigmatocystin	$C_{18}H_{12}O_6$	P56	324



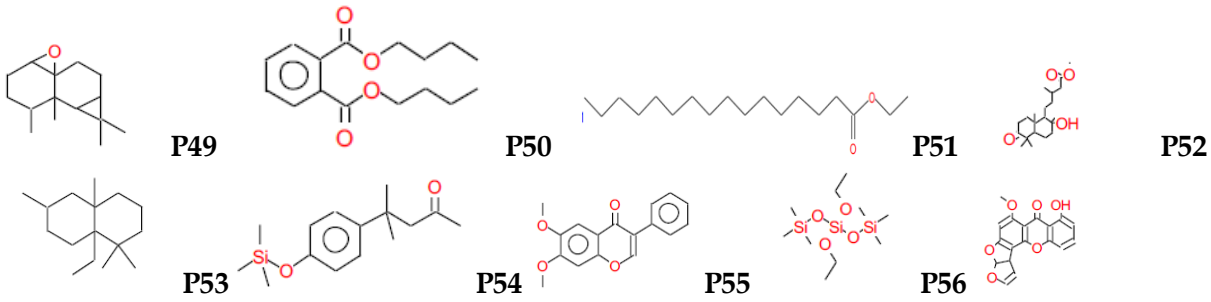


Table 7: Bioactivity of Compounds Identified in Ethanolic Extract of *Bridelia ferriginea* Bark by GC-MS

Name of Compound	Bioactivity	Reference
4-Vinyl-imidazole	Antifungal	Yang *et al.* (2018)
5-Vinyl-pyrazole	antitumor, anti-inflammatory, and antifungal	Wu *et al.* (2015); Nayak *et al.* (2020)
1,3,8-p-Menthatriene	Antimicrobial	Neoh *et al.* (2008)
o-Cymene	antimicrobial and antioxidant	Neoh *et al.* (2008)
n-Butylbenzene	cytotoxicity	Neoh *et al.* (2008)
2,6-Diaminopimelic acid	Antibacterial	Neoh *et al.* (2008).
1-Tridecene	Bio pesticide	Neoh *et al.* (2008)
3-Propylglutaric acid	Neuroprotective	Neoh *et al.* (2008)
PET-cGMP	cellular signaling associated with vasodilation and neurotransmission	Neoh *et al.* (2008)
Decynediol, tetramethyl, di(2-hydroxyethyl) ether	Unknown bioactivity	
Isophthalic acid, 2-formylphenyl propyl ester	anti-inflammatory	Neoh *et al.* (2008)
Phytol	antioxidant and anti-inflammatory	Neoh *et al.* (2008)
Silane, [(6a,7,8,10a-tetrahydro-6,6,9-trimethyl-3-pentyl-6H-dibenzo[b,d]pyran -1,8-diyl)bis(oxy)]bis[dimethylphenyl-, [6ar-(6aα,8β,10aβ)]-	Therapeutic	Neoh *et al.* (2008)
Decanoic acid, 2-methyl-	Antimicrobial	Neoh *et al.* (2008)
Tridecanoic acid, methyl ester	Antimicrobial	Neoh *et al.* (2008)
Benzene, 2-methoxy-4-methyl-1-(1-methylethyl)-	insect repellent	Neoh *et al.* (2008)
Oxirane, [(hexadecyloxy)methyl]-	unknown bioactivity	
Proadifen	drug metabolism and detoxification	Neoh *et al.* (2008)
Silicic acid, diethyl bis(trimethylsilyl) ester	Therapeutic	Neoh *et al.* (2008)
Methyl stearate	Antimicrobial	Neoh *et al.* (2008)

Ethyl [5-hydroxy-1-(6-methoxy-4-methyl-3-quinolinyl)-3-methyl-1H-pyrazol-4-yl]acetate	Antitumor	Neoh *et al.* (2008)
Isoproturon	Herbicide	Neoh *et al.* (2008)
Genistin	antioxidant and anti-inflammatory	Neoh *et al.* (2008)
8-Isopropyl-5-methyl-5,6,7,8-tetrahydro-2,4-quinazolinedione	anti-inflammatory	Neoh *et al.* (2008)

Table 8: Bioactivity of Compounds Identified in Ethanolic Extract of *Khaya senegalensis* Bark by GC-MS

Name of Compound	Bioactivity	Reference
Ethanol	Antimicrobial	Mujeeb *et al.* (2014)
L-Lactic acid	Antimicrobial	Mujeeb *et al.* (2014)
Trinexapac-ethyl	plant growth regulator	Mujeeb *et al.* (2014)
7-Methoxyflavanone	antioxidant, anti-inflammatory, and anticancer	Mujeeb *et al.* (2014)
Temefos	insecticide	Mujeeb *et al.* (2014)
*(2S,4S)-2,4-Dimethylheptanedioic acid dimethyl ester	Synthesis of biodegradable polymers and as a plasticizer. enhance the mechanical properties of polymeric materials	Mujeeb *et al.* (2014)
2,6-Dimethyl-3-thioxo-5-oxo-2,3,4,5-tetrahydro-1,2,4-triazine	antimicrobial	Mujeeb *et al.* (2014)
3,5-Dimethoxycinnamic acid	antioxidant and anti-inflammatory	Mujeeb *et al.* (2014)
1-Octanone, 1-(2-octylcyclopropyl)-	Antimicrobial and flavouring agent	Mujeeb *et al.* (2014)
Tetradecanoic acid, 10,13-dimethyl-, methyl ester	Antimicrobial	Ferdosi *et al.* (2022)
*Dibutyl phthalate	Plasticizer and endocrine disrupting effect	Mujeeb *et al.* (2014)
Dodecanoic acid, ethyl ester	antimicrobial properties and food preservation	Ferdosi *et al.* (2022)
Trimethyl[4-(2-methyl-4-oxo-2-pentyl)phenoxy]silane	Therapeutic	Mujeeb *et al.* (2014)
Palmitoyl ceramide	anti-inflammatory	Mujeeb *et al.* (2014)
Imipramine	antidepressant	Mujeeb *et al.* (2014)
*1,2,4-Benzenetricarboxylic acid, 1,2-dimethyl ester	Plasticizer	Mujeeb *et al.* (2014)
Quinomethionate	Neurotoxic	Mujeeb *et al.* (2014)
2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-	Antioxidant	Mujeeb *et al.* (2014)

Key:*Phthalates contamination

Table 9: Bioactivity of Compounds Identified in Ethanolic Extract of *Psidium guajava* Leaves by GC-MS

Name of Compound	Bioactivity	Reference
α-Bisabolol	anti-inflammatory, antimicrobial, and wound-healing	Nabi *et al.* (2022)
Methyl 4-hydroxybenzoate	antimicrobial	Qadeer *et al.* (2007)
Dioxatricyclo[5.1.0.0(3,5)]octane, 1-methyl-5-(1-methylethyl)-, (1α,3α,5α, 7α)-	Unknown bioactivity	
Germacrene D	antimicrobial and insecticidal	Bauta *et al.* (2003)
6-(3-Methyl-3-cyclohexenyl)-2-methyl-2,6-heptadienol	flavoring agent and its sensory properties	(Krishnamoorthy and Paulsamy, 2014)
Aromandendrene	antimicrobial	Bauta *et al.* (2003)
cis-muurola-3,5-diene	natural insect repellent	Nouni, (2023)
cis-muurola-4(14),5-diene	insecticidal	Nouni, (2023)
Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	Unknown bioactivity	
1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3α,3aβ, 7β,8aα)]-	Unknown bioactivity	
Cedrene	antimicrobial and insecticidal	Bauta *et al.* (2003)
3-Phosphoglyceric acid	metabolic intermediate	Rani and Kumari, (2014)
2-Phosphoglyceric acid	energy metabolism	Rani and Kumari, (2014)
3-Buten-2-one, 4-(2,5,6,6-tetramethyl-1-cyclohexen-1-yl)-	flavoring and fragrance	Hu *et al.* (2023)
3H-1,2,4-Triazol-3-one, 4-[4-[(2-aminoethyl)amino]phenyl]-2,4-dihydro-2-[[4-(trifluoromethyl)phenyl]methyl]-	antimicrobial agent	Müller and Applebyki, (2011)
Ethanone, 1-(1,3a,4,5,6,7-hexahydro-4-hydroxy-3,8-dimethyl-5-azulenyl)-	Unknown bioactivity	
1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane	Unknown bioactivity	
Cyclohexene, 3-methyl-6-(1-methylethenyl)-, (3R-trans)-	Unknown bioactivity	
Dihydro-cis-α-copaene-8-ol	Antimicrobial	Bauta *et al.* (2003)
Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1α, 7β,8aα)]-	Unknown bioactivity	
Quinic acid	antioxidant and anti-inflammatory	Hamama *et al.* (2018)
2,3-Dehydro-4-oxo-β-ionone	flavoring and fragrance	El-Barbary *et al.* (2001)
Dihydro-cis-α-copaene-8-ol	Insecticidal and antimicrobial	Sun *et al.* (2020); Mehrzadeh, (2024)

2-(3-Methyl-but-1-ynyl)-cyclohexene-1-carboxaldehyde	flavoring agents and in fragrances.	Mehrzadeh, (2024)
Viridiflorol	Antimicrobial	Bauta *et al.* (2003)
Aromandendrene	Antimicrobial, antioxidant and anti-inflammatory	Mehrzadeh, (2024)
all trans-Retinal	vision and retinal health	Makki *et al.* (2015)
Cholecalciferol	immune function and cellular growth regulation	Газиева and Кравченко, (2014)
Benzene, 2-methoxy-4-methyl-1-(1-methylethyl)-	Unknown bioactivity	
cis-3-Methyl-β-methyl-β-nitrostyrene	Unknown bioactivity	
2H-1-Benzopyran, 3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl-, (2α,4aα,8aα)-	Unknown bioactivity	
2-Butenal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	antioxidant properties	Mal *et al.* (2020)
Citronellic acid	insect repellent	Makki *et al.* (2014)
Sinapic acid	antioxidant and anti-inflammatory	Esseffar *et al.* (2008)
Photocitral A	Unknown bioactivity	
1,4-Methano-3-benzoxepin-5(4H)-one, 1,2,7,8,9,9a-hexahydro-7,10-dihydroxy-2,2,9,9a-tetramethyl-	anti-inflammatory or analgesic	López-Sánchez, (2024)
Cyclohexene-1-methanol, α,4-dimethyl-α-(4-methyl-3-pentenyl)-	antimicrobial or antifungal properties	Ganji *et al.* (2013)
trans-3-Ethoxy-b-methyl-b-nitrostyrene	therapeutic applications	Silva *et al.* (2010)
Phosphoric acid, diethyl 1-methylenepropyl ester	Unknown bioactivity	
2-Cyclopenten-1-one, 4-hydroxy-3-methyl-2-(2,4-pentadienyl)-	Unknown bioactivity	
1-Thienylcyclohexene	Unknown bioactivity	
Calarene epoxide	Unknown bioactivity	
*Dibutyl phthalate	Plasticizer, endocrine-disrupting effects	Mujeeb *et al.* (2014)
Hexadecanoic acid, ethyl ester	antioxidant, anti-inflammatory, and anticancer activities	Nabi *et al.* (2022)
1-Naphthalenepentanoic acid, decahydro-2-hydroxy-β,2,5,5,8a-pentamethyl-6 -oxo-, methyl ester	anti-inflammatory or analgesic	Vavasori *et al.* (2003)
Decahydro-8a-ethyl-1,1,4a,6-tetramethylnaphthalene	Unknown bioactivity	
Trimethyl[4-(2-methyl-4-oxo-2-pentyl)phenoxy]silane	Unknown bioactivity	
4H-1-Benzopyran-4-one, 6,7-dimethoxy-3-phenyl-	Unknown bioactivity	

Silicic acid, diethyl bis(trimethylsilyl) ester	Unknown bioactivity
Sterigmatocystin	Unknown bioactivity

Key:*Phthalates contamination

DISCUSSION

The analysis of the extract using Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy (ATR- FTIR) and Gas Chromatography-Mass Spectrometry (GC-MS) provides valuable insights into the chemical composition and functional groups present in *B. ferruginea*, *K. senegalensis* and *P. guajava* extracts. The ATR-FTIR results for *B. ferruginea* indicate several peak values, including 3198.1 cm^{-1}, 2105.9 cm^{-1}, 1908.4 cm^{-1}, 1602.8 cm^{-1}, 1520.8 cm^{-1}, 1438.8 cm^{-1}, 1319.5 cm^{-1}, 1282.2 cm^{-1}, 1196.5 cm^{-1}, 1103.3 cm^{-1}, and 1028.7 cm^{-1} as shown in Figure 1. These peaks correspond to various functional groups which can be associated with the molecular structure of the compounds present in the extract as shown in Table 1.The peak at 3198.1 cm^{-1} typically indicates the presence of hydroxyl (-OH) groups or amines, which are common in many organic compounds, suggesting potential antioxidant properties (Thummajitsakul *et al.,* 2022). The peaks around 1602.8 cm^{-1} and 1520.8 cm^{-1} are often attributed to aromatic C=C stretching vibrations, indicating the presence of aromatic compounds, which can contribute to the extract's biological activities (Thummajitsakul and Silprasit, 2022). In the finger print region, the peaks in the region of 1100-1300 cm^{-1} are generally associated with C-O stretching vibrations, which further support the presence of alcohols, phenols, or ethers (Farooq *et al.,* 2022).

In conjunction with the ATR-FTIR analysis, the GC-MS results revealed a diverse array of compounds identified in the extract, including 4-Vinyl-imidazole, 5-Vinyl-pyrazole, o-Cymene, and Phytol, among others. These compounds have been documented for various biological activities as shown in Table 4. For instance, o-Cymene is known for its antimicrobial and anti-inflammatory properties (Starlin *et al.,* 2019). Phytol, a common phytochemical, has been associated with antioxidant and anti-inflammatory effects, which may enhance the therapeutic potential of the extract (Chiguvare *et al.,* 2016). The presence of fatty acids such as Decanoic acid and Tridecanoic acid, as well as esters like Methyl stearate, suggests that the extract may also possess lipid-regulating properties, which could be beneficial in various health contexts (Uraku, 2015).

The identification of compounds such as 2,6-Diaminopimelic acid and 3-Propylglutaric acid indicates that the extract may have potential applications in medicinal chemistry, particularly in the development of new therapeutic agents (Nattala, 2019). Additionally, the presence of Isoproturon and Genistin suggests that the extract may exhibit herbicidal and antioxidant activities, respectively (Rădulescu *et al.,* 2020).

For *K. senegalensis*, the ATR- FTIR results reveal several significant peak values, including 3209.2 cm^{-1}, 2922.2 cm^{-1}, 1699.7 cm^{-1}, 1602.8 cm^{-1}, 1520.8 cm^{-1}, and 1200-1300 cm^{-1} as shown in Figure 2. Each of these peaks corresponds to specific functional groups that are indicative of the chemical nature of the compounds present in the extract as shown in Table 2.The peak at 3209.2 cm^{-1} is typically associated with O-H stretching vibrations, which suggests the presence of alcohols or phenolic compounds (Morah, 2023). This is further supported by the peak at 1699.7 cm^{-1}, which is indicative of carbonyl (C=O) stretching, commonly found in carboxylic acids and esters (Keskes *et al.,* 2016). The peaks around 1602.8 cm^{-1} and 1520.8 cm^{-1} correspond to aromatic C=C stretching, indicating the presence of aromatic compounds that

may contribute to the extract's bioactivity (Thummajitsakul and Silprasit, 2022). Additionally, in the finger print region, the peaks in the region of 1200-1300 cm^{-1} are often attributed to C-O stretching vibrations, which are characteristic of ethers and alcohols (Yilwa *et al.,* 2022). The presence of these functional groups suggests that the extract may possess various biological activities, including antioxidant and anti-inflammatory properties.

The GC-MS analysis identifies a diverse range of compounds within the extract, including Ethanol, L-Lactic acid, and 7-Methoxyflavanone, among others. Ethanol is the solvent used in the extraction process, and its presence may indicate the extraction method employed (Kamble and Gaikwad, 2016). L-Lactic acid is known for its role in metabolic processes and may contribute to the extract's potential health benefits (Yusof *et al.,* 2014). The identification of 7-Methoxyflavanone, a flavonoid, suggests that the extract may possess antioxidant properties, as flavonoids are well-documented for their ability to scavenge free radicals (Dike, 2023).

Other notable compounds identified include 3,5-Dimethoxycinnamic acid, which is recognized for its anti-inflammatory and antioxidant activities (Thummajitsakul *et al.,* 2022). The presence of fatty acids such as Tetradecanoic acid and Dodecanoic acid, along with their methyl esters, indicates that the extract may have lipid-regulating properties, which could be beneficial in various health contexts (Ovais *et al.,* 2018). The presence of phthalates such as (2S,4S)-2,4-Dimethylheptanedioic acid dimethyl ester, Dibutyl phthalate and 1,2,4-Benzenetricarboxylic acid, 1,2-dimethyl ester in the plant extract suggests a possibility of contamination from the plastic container used in storing the extract or from the soil environment where the plant was sourced. These plants uptake these toxic chemicals through the roots from dissolved plastics with no covalent bond (Omidpanah *et al.,* 2018). Furthermore, compounds like Imipramine, an antidepressant, suggest potential neuroactive properties of the extract, which may warrant further investigation (Mohammed *et al.,* 2023). For *P. guajava*, the ATR-FTIR results reveal several key peak values, including 3235.3 cm^{-1}, 2918.5 cm^{-1}, 2851.4 cm^{-1}, 2113.4 cm^{-1}, 1684.8 cm^{-1}, and 1606.5 cm^{-1}, 1520.8 $cm^{-1,}$ 1442.5 $cm^{-1,}$ 1315.8 cm^{-1} , 1282.2 cm^{-1}, 1200.2 cm^{-1}, 1103.3 cm^{-1}, 1032.5 cm^{-1}, and 764.1 cm^{-1}as shown in Figure 3. Each of these peaks corresponds to specific functional groups that are indicative of the chemical nature of the compounds present in the extract as shown in Table 3.

The peak at 3235.3 cm^{-1} is typically associated with O-H stretching vibrations, suggesting the presence of hydroxyl groups, which are common in alcohols and phenolic compounds (Sobha *et al.,* 2017). This is further supported by the peak at 1684.8 cm^{-1}, indicative of carbonyl (C=O) stretching, often found in carboxylic acids and esters (Ram *et al.,* 2020). The peaks around 1606.5 cm^{-1} correspond to aromatic C=C stretching, indicating the presence of aromatic compounds that may contribute to the extract's antioxidant and anti-inflammatory properties (Elya *et al.,* 2012). Additionally, the peaks in the finger print region of 1200-1300 cm^{-1} are typically attributed to C-O stretching vibrations, characteristic of ethers and alcohols (Rasheed and Jabeen, 2022). The presence of these functional groups suggests that the extract may possess various biological activities, including potential therapeutic effects.

The GC-MS analysis identifies a diverse array of compounds within the extract, including α-Bisabolol, Methyl 4-hydroxybenzoate, and Germacrene D, among others. α-Bisabolol is known for its anti-inflammatory and antimicrobial properties, making it a valuable component for potential therapeutic applications (Pakkirisamy *et al.,* 2017). Methyl 4-hydroxybenzoate, also known as methylparaben, is widely used as a preservative and has been shown to possess antimicrobial properties (Mubeen *et al.,* 2022). The presence of

Germacrene D, a sesquiterpene, is notable as it is recognized for its potential anti-inflammatory and anticancer activities (Krishnamoorthy and Paulsamy, 2014).

Just like *K. senegalensis* extract, *P. guajava* extract also contained Dibutyl phthalate (phthlate contamination). Other significant compounds identified include various naphthalene derivatives and cyclohexene compounds, which may contribute to the extract's aromatic profile and potential biological activities (Gnanakani *et al.,* 2019). For instance, the presence of 3-Phosphoglyceric acid and Quinic acid suggests metabolic roles that could be beneficial in various physiological processes (Parekh and Chanda, 2010). Furthermore, the identification of compounds like Sinapic acid and all trans-Retinal indicates potential antioxidant properties, as these compounds are known for their ability to scavenge free radicals (Subramanian *et al.,* 2016).

CONCLUSION

The combined ATR-FTIR and GC-MS analyses of *B. ferruginea*, *K. senegalensis* and *P. guajava* provide details of their characterization, revealing a rich profile of functional groups, bioactive compounds and their respective bioactivity. This information is crucial for understanding the potential health benefits and applications of these extracts in medicinal and therapeutic contexts. The presence of various bioactive compounds suggests that the extracts may have significant pharmacological potential, warranting further investigation into its therapeutic applications in its use for treatment of gastro intestinal diseases.

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