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# Comparative Phytochemical, FTIR and GC–MS Characterization of Aqueous and Ethanolic Leaf Extracts of *Azadirachta indica*, *Psidium guajava* and *Ocimum gratissimum*

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**ABSTRACT**
Medicinal plants are important sources of bioactive compounds with potential pharmaceutical applications. This study comparatively evaluated the extraction yield, phytochemical composition, Fourier Transform Infrared (FTIR) characteristics, and Gas Chromatography–Mass Spectrometry (GC–MS) profiles of aqueous and ethanolic leaf extracts of *Azadirachta indica*, *Psidium guajava*, and *Ocimum gratissimum* were collected from Kaduna state, Nigeria. Leaf powders were extracted separately by cold maceration using distilled water and ethanol. The extracts were subjected to qualitative and quantitative phytochemical analyses, FTIR spectroscopy, and GC–MS characterization using standard analytical procedures. Ethanolic extraction produced higher yields (9.4–11.3%) than aqueous extraction (7.6–8.2%) across all plant species. Phenols, flavonoids, tannins, alkaloids, saponins, and terpenoids were detected in all extracts, although their concentrations varied among plant species and extraction solvents. *Ocimum gratissimum* exhibited the highest phenolic (11.21 ± 1.62 mg/g) and flavonoid (6.13 ± 1.35 mg/g) contents, whereas *Psidium guajava* contained the highest tannin concentration (5.74 ± 1.72 mg/g). FTIR analysis confirmed the presence of characteristic functional groups associated with phenolics, alcohols, esters, aromatic compounds, and other oxygenated metabolites. GC–MS analysis identified diverse bioactive constituents, predominantly fatty acids, fatty acid derivatives, long-chain hydrocarbons, esters, and related compounds with documented antimicrobial and antioxidant activities. The observed variations in extraction yield and phytochemical contents among plant species and extraction solvents were statistically significant ($p < 0.05$) based on one-way ANOVA. The findings demonstrate that ethanol is a more efficient extraction solvent than water and highlight the rich phytochemical diversity of these medicinal plants.

**Keywords:** Medicinal Plant, Extraction Yield, Ethanol, Phytochemicals, Bioactive Compound

## INTRODUCTION

Medicinal plants remain an important source of biologically active compounds and continue to play a significant role in healthcare systems worldwide. In recent years, interest in plant-derived therapeutics has increased because of the growing prevalence of antimicrobial resistance (AMR), reduced effectiveness of conventional antibiotics, and the need for safer and more sustainable alternatives. Secondary metabolites synthesized by medicinal plants exhibit a wide range of biological activities, including antimicrobial, antioxidant, anti-inflammatory, antiviral and anticancer effects, making them valuable candidates for pharmaceutical and nutraceutical development (Angelini, 2024; Liu et al., 2024).

Among medicinal plants with established ethnomedicinal importance are *Azadirachta indica*. (neem), *Psidium guajava* (guava), and *Ocimum gratissimum* L. (African basil). These species are widely distributed throughout tropical and subtropical regions, where they have long been used in traditional medicine to manage infectious diseases, gastrointestinal disorders, inflammatory conditions, respiratory ailments and skin infections. Their therapeutic properties have been attributed to diverse phytochemicals, including phenolic compounds, flavonoids, tannins, alkaloids, saponins, terpenoids and essential oils. These metabolites exert biological activities through multiple mechanisms, such as disruption of microbial cell membranes, inhibition of essential enzymes, interference with nucleic acid synthesis, modulation of oxidative stress and suppression of microbial virulence factors (Khameneh et al., 2021; Abdul Mueed et al., 2023).

The medicinal value of these plants is largely attributed to their diverse phytochemicals. *Azadirachta indica* is recognized for its antimicrobial, anti-inflammatory, antioxidant, and antimalarial activities, mainly associated with compounds such as azadirachtin, nimbin, and quercetin. *Psidium guajava* has demonstrated antibacterial, antioxidant, antidiarrhoeal, and wound-healing properties linked to polyphenols including quercetin, catechin, gallic acid, and ellagic acid. Likewise, *Ocimum gratissimum* exhibits antimicrobial, antifungal, anti-inflammatory, and antioxidant activities, largely due to eugenol, thymol, flavonoids, and tannins. Together, these findings underscore the therapeutic potential of the three species and the importance of further phytochemical characterization.

The quantity and composition of phytochemicals recovered from medicinal plants depend on several factors, including plant species, geographical origin, and environmental conditions, harvesting period, post-harvest handling and extraction procedures. Among these factors, solvent selection is particularly important because extraction efficiency is strongly influenced by solvent polarity. Water preferentially extracts highly polar constituents, whereas ethanol is capable of dissolving both polar and moderately non-polar

compounds, resulting in the recovery of a broader spectrum of secondary metabolites. Consequently, comparative evaluation of aqueous and ethanolic extracts provides useful information for selecting appropriate extraction systems and maximizing the recovery of biologically active compounds (Altemimi et al., 2023; Plaskova & Mlček, 2023).

A comprehensive evaluation of medicinal plant extracts requires analytical techniques that provide different but complementary information. Preliminary phytochemical screening identifies the major classes of bioactive compounds present in the extracts. FTIR spectroscopy then provides a rapid chemical fingerprint by detecting the functional groups associated with these compounds, whereas GC–MS separates and identifies individual volatile and semi-volatile constituents based on their mass spectral characteristics. By combining FTIR and GC–MS, both the overall chemical composition and specific bioactive constituents can be characterized more effectively than with either technique alone. This integrated approach improves phytochemical profiling, supports quality assessment and standardization, and facilitates the identification of compounds with potential pharmacological importance (Lepe de Alba et al., 2023; Tian et al., 2024).

In Northern Nigeria, including Kaduna State, these medicinal plants are widely used in traditional healthcare because they are readily available, affordable, and deeply rooted in local ethnomedicinal practices. *Azadirachta indica* leaves are commonly used to prepare herbal remedies for malaria, fever, skin infections, and gastrointestinal disorders. *Psidium guajava* leaves are traditionally employed in the treatment of diarrhoea, dysentery, wounds, cough, and oral infections, while *Ocimum gratissimum* is frequently used to manage respiratory infections, stomach ailments, fever, headaches, and various skin diseases. The continued reliance on these plants by local communities highlights their medicinal importance and emphasizes the need for scientific validation of their phytochemical composition and bioactive constituents. Such information is essential for supporting their safe use, standardization, and potential development into evidence-based herbal medicines.

Although *Azadirachta indica*, *Psidium guajava*, and *Ocimum gratissimum* have each been extensively studied for their phytochemical composition and medicinal properties, direct comparisons of these three species under the same experimental conditions remain scarce. Most previous studies have focused on individual plant species or compared extraction solvents within a single species, making it difficult to evaluate differences in extraction efficiency and chemical composition across these medicinal plants. In addition, only a few studies have combined qualitative and quantitative phytochemical analyses with FTIR and GC–MS characterization using standardized extraction procedures. Information on these plants collected from Northern Nigeria is particularly limited, even though geographical and environmental factors can influence the type and abundance of phytochemicals present. Consequently, there is still a need for comprehensive comparative studies that generate baseline data to support quality control, standardization, and future pharmacological research.

Therefore, this study comparatively evaluated the extraction yield, qualitative and quantitative phytochemical composition, FTIR characteristics and GC–MS profiles of aqueous and ethanolic leaf extracts of *Azadirachta indica*, *Psidium guajava* and *Ocimum gratissimum*. The findings provide comprehensive chemical information on these medicinal plants, establish baseline data for their standardization and quality assessment, and contribute to the growing body of evidence supporting their potential use as sources of bioactive compounds for future pharmaceutical applications.

## MATERIALS AND METHODS

### Study Area and Plant Collection

Fresh leaves of *Azadirachta indica*, *Psidium guajava*, and *Ocimum gratissimum* were collected from the Nigerian Defence Academy (NDA), Kaduna State, Nigeria, in January 2026. The plant samples were authenticated by a taxonomist at the Herbarium, Department of Biological Sciences, NDA, Kaduna. Voucher specimens were prepared and deposited at the herbarium for future reference. The corresponding voucher numbers were *A. indica* (NDA/BIOH/202652), *O. gratissimum* (NDA/BIOH/202653), and *P. guajava* (NDA/BIOH/202654).

### Preparation of Plant Materials and Extraction

The leaves were washed thoroughly with tap water followed by distilled water, shade-dried at room temperature until a constant weight was obtained, and pulverized into fine powder using a laboratory grinder. The powdered samples were stored in clean, airtight containers until extraction.

The extraction procedure employed 500 g of powdered leaf material in 2.5 L of solvent (1:5 w/v) to ensure complete immersion of the plant material throughout the extraction process. This solvent-to-sample ratio was selected because it provides sufficient solvent to facilitate efficient diffusion and dissolution of bioactive phytochemicals while minimizing solvent saturation during maceration. Maintaining a 1:5 (w/v) ratio is a widely adopted approach in phytochemical extraction studies, as it promotes optimal mass transfer and improves extraction efficiency without excessive solvent consumption. To reduce solvent loss and protect the extracts from light, the containers were tightly covered throughout the extraction period. To minimize the degradation of light-sensitive phytochemicals, the extraction containers were tightly covered and kept under light-protected conditions throughout the 72-hour maceration period. The powdered leaf samples were macerated in the respective solvents for 72 hours to ensure adequate contact between the solvent and the plant material, allowing for the efficient extraction of bioactive constituents. This extraction period has been widely adopted in phytochemical studies because it provides sufficient time for the diffusion of phytochemicals into the solvent while minimizing the degradation of heat-sensitive compounds.

After extraction, the mixtures were filtered through Whatman No. 1 filter paper. The filtrate was then centrifuged at 10,000 rpm for 10 minutes at room temperature (approximately 25°C) to remove suspended particles and obtain a clear supernatant. The resulting supernatant was concentrated using a rotary evaporator for the ethanolic extracts and a water bath maintained at 45°C for the aqueous extracts until crude extracts were obtained. The ethanolic extracts were concentrated under reduced pressure using a rotary evaporator at 40°C, whereas the aqueous extracts were concentrated in a water bath at 50°C (or freeze-dried, where applicable).

The dried extracts were then weighed to determine the percentage extraction yield and stored in airtight containers at 4°C until further phytochemical and antimicrobial analyses. The concentrated extracts were dried to constant weight, transferred into sterile amber bottles, and stored at 4°C until analysis.

**Determination of Extraction Yield**

The percentage extraction yield of each extract was determined using:

Extraction yield (%) =Weigh of dried extract (g)/Weight of dried plant material (g) x100

Where:

Weight of dried extract = the mass (g) of the concentrated extract obtained after solvent evaporation.

Weight of dried plant material = the initial mass (g) of the dried powdered plant used for extraction.

**Qualitative Phytochemical Screening**

Qualitative phytochemical screening was carried out using standard procedures described by Harborne (1973), Sofowora (1993), Obadoni and Ochuko (2001), and Lakache (2016). The extracts were screened for alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, phytosterols, cardiac glycosides, anthraquinones, flavonols, coumarins, quinones, resins, amino acids, chalcones, phlobatannins, triterpenoids, and vitamin A based on characteristic colour changes or precipitate formation.

**Quantitative Phytochemical Determination**

The concentrations of total phenols, flavonoids, tannins, alkaloids, saponins, and terpenoids were determined using validated standard procedures. Total phenolic content was measured using the Folin–Ciocalteu assay with gallic acid as the calibration standard, and absorbance was recorded at 765 nm. Flavonoid content was estimated by the aluminium chloride colorimetric method using quercetin as the reference standard, with absorbance measured at 415 nm. Tannin content was determined using the Folin–Denis method with tannic acid as the standard and absorbance read at 760 nm. Alkaloids, saponins, and terpenoids were quantified using established gravimetric methods based on selective precipitation and solvent extraction. All measurements were carried out in triplicate, and the results were expressed as mean ± standard deviation.

**Fourier Transform Infrared (FTIR) Analysis**

Functional groups present in the ethanolic extracts were characterized using a Fourier Transform Infrared (FTIR) spectrometer over the spectral range of 4000–400 $cm^{-1}$. The observed absorption bands were interpreted by comparison with standard reference spectra to identify characteristic functional groups associated with the phytochemical constituents.

**Gas Chromatography-Mass Spectrometry (GC-MS)**

The chemical composition of the ethanolic extracts was analysed using Gas Chromatography–Mass Spectrometry (GC–MS). Separation was achieved on a capillary column with helium as the carrier gas under optimized operating conditions. Compounds were identified by comparing their mass spectra with those in the National Institute of Standards and Technology (NIST) Mass Spectral Library, while relative abundances were calculated from chromatographic peak areas and expressed as percentages of the total ion chromatogram.

**Statistical Analysis**

All experiments were carried out in triplicate, and data are presented as mean ± standard deviation (SD). Statistical analyses were performed using IBM SPSS Statistic. Differences among means were analysed by one-way analysis of variance (ANOVA), followed by Tukey's post hoc multiple comparison test. Statistical significance was established at $p < 0.05$.

## RESULTS AND DISCUSSION

### Results

***Extraction Yield of the Leaf Extracts***

The extraction yields of the aqueous and ethanolic leaf extracts of *Azadirachta indica*, *Psidium guajava*, and *Ocimum gratissimum* are presented in Table 1. Ethanolic extraction consistently produced higher yields than aqueous extraction for all three plant species. Among the ethanolic extracts, *P. guajava* recorded the highest extraction yield (11.3%), followed by *A. indica* (9.9%) and *O. gratissimum* (9.4%). For the aqueous extracts, *O. gratissimum* (7.8%) and *P. guajava* (7.6%) produced comparable yields, while *A. indica* recorded the lowest yield.

**Table 1: Percentage Extraction Yield of Aqueous and Ethanolic Leaf Extracts of *Azadirachta indica*, *Psidium guajava*, and *Ocimum gratissimum***

| Plant | Solvent weight of plant | Weight of extract | %yield | Colour | Texture |
|---|---|---|---|---|---|
| *A. indica* Aqueous | 500 | 32.4 | 6.48 | Dark brown | Gummy |
| *A. indica* Ethanol | 500 | 49.6 | 9.92 | Dark green | Sticky |
| *P. guajava* Aqueous | 500 | 38.4 | 7.68 | Dark brown | Gummy |
| *P.guajava* Ethanol | 500 | 56.4 | 11.28 | Dark green | Waxy |
| *O. gratissimum* AQ | 500 | 39.2 | 7.84 | Brownish black | Sticky |
| *O. gratissimum* ETH | 500 | 47.2 | 9.44 | Dark green | Waxy |

***Qualitative and Quantitative Phytochemical Composition***

The qualitative and quantitative phytochemical compositions of the aqueous and ethanolic leaf extracts are presented in Table 2**.** Qualitative screening revealed the presence of tannins, alkaloids, phenols, saponins, flavonols/flavones, and coumarins in all extracts. In contrast, anthocyanins and chalcones were not detected in any of the samples, whereas phytosterols, cardiac glycosides, quinones, resins, amino acids, phlobatannins, and triterpenoids varied according to plant species and extraction solvent. Phytosterols were detected in the ethanolic extracts of *P. guajava* and *O. gratissimum*, whereas they were not detected in the corresponding aqueous extracts. This may be associated with the predominantly lipophilic nature of phytosterols and the greater ability of ethanol to extract relatively non-polar constituents. However, non-detection in the aqueous extracts does not necessarily indicate complete absence of phytosterols, as the result may also be influenced by the sensitivity of the qualitative screening procedure.

Quantitative analysis showed marked differences in phytochemical concentrations among the extracts. The ethanolic extract of *O. gratissimum* contained the highest phenolic (11.21 ± 1.62 mg/g) and flavonoid (6.13 ± 1.35 mg/g) contents, while the ethanolic extract of *P. guajava* recorded the highest tannin concentration (5.74 ± 1.72 mg/g). The aqueous extract of *A. indica* exhibited the highest alkaloid

content (5.54 ± 1.25 mg/g). Saponins and terpenoids were detected in all extracts, although their concentrations varied among the plant species.

**Table 2: Qualitative and Quantitative Phytochemical Composition of Aqueous and Ethanolic Leaf Extracts of *Azadirachta indica*, *Psidium guajava*, and *Ocimum gratissimum***

| Phytochemical | *A. indica*AQ | *A. indica*ETH | *P. guaja*AQ | *P. guajava*ETH | *O. gra*AQ | *O. gra*ETH |
|---|---|---|---|---|---|---|
| Tannins | +(1.26±0.15) | +(3.85±1.05) | +(1.18±0.12) | +(5.74±1.72) | +(2.21±0.25) | +(4.02±0.78) |
| Alkaloids | +(5.54±1.25) | +(2.75±0.06) | +(1.83±1.36) | +(2.20±0.06) | +(1.39±1.12) | +(1.63±1.14) |
| Phenols | +(9.10±1.20) | +(8.62±1.25) | +(8.71±2.02) | +(8.43±1.09) | +(4.01±1.20) | +(11.21±1.62) |
| Saponins | +(2.37±0.91) | +(4.17±1.20) | +(2.08±0.24) | +(1.67±0.66) | +(3.23±1.14) | +(2.08±1.20) |
| Terpenoids | +(1.02±1.14) | +(0.68±0.14) | +(0.86±0.24) | +(1.82±1.64) | +(0.25±0.14) | +(1.22±1.14) |
| Flavonoids | +(1.01±0.18) | +(2.37±1.14) | +(2.30±0.25) | +(3.26±1.43) | +(1.15±1.05) | +(6.13±1.35) |
| Phytosterols | - | - | - | + | - | + |
| Vitamin A | + | - | + | - | - | - |
| Cardiac glycoside | - | + | - | + | + | + |
| Anthraquinones | + | + | - | + | - | - |
| Anthocyanins | - | - | - | | - | - |
| Phlobotanins | - | + | + | + | - | + |
| Flavonols/flavones | + | + | + | + | + | + |
| Coumarins | + | + | + | + | + | + |
| Quinolones | - | + | - | + | + | - |
| Resins | - | - | - | + | - | + |
| Amino acids | - | - | + | + | - | - |
| Chalcones | - | - | - | - | - | - |
| Triterpenoids | + | - | - | + | - | + |

LEGEND: Qualitative phytochemical screening — presence/absence (+/−)
Quantitative phytochemical analysis **—** values expressed in appropriate units
Note: (+) = detected/present; (−) = not detected/absent. The symbols indicate only the presence or absence of the tested phytochemical and do not represent the intensity or concentration of the reaction. Quantitative results are expressed as mean ± standard deviation

***FTIR Characterization of Ethanolic Leaf Extracts***

The FTIR spectra of the ethanolic extracts are summarized in Table 3. All three plant species exhibited characteristic absorption bands corresponding to hydroxyl (O–H), carbonyl (C=O), aromatic (C=C), and carbon–oxygen (C–O) functional groups. Minor differences in peak positions and intensities were observed among the extracts, indicating variations in their chemical compositions while demonstrating the presence of structurally related phytochemical constituents.

**Table 3: Comparative FTIR Absorption Bands and Functional Group Assignments of the Ethanolic Leaf Extracts of *Azadirachta indica, Psidium guajava* and *Ocimum gratissimum***

| Functional Group | Vibration Type | *A. indica* ($cm^{-1}$) | *P. guajava* ($cm^{-1}$) | *O. gratissimum* ($cm^{-1}$) | Probable Phytochemical/Compound |
|---|---|---|---|---|---|
| O–H | Stretching | 3412.13 | 3419.90 | 3412.19 | Alcohols, phenols, flavonoids |
| =C–H | Stretching | — | — | 3113.21 | Aromatic compounds |
| C–H ($sp^3$) | Stretching | 2831.10 | — | 2985.04 | Alkanes, terpenoids |
| C–H | Stretching | 2738.11 | — | — | Aldehydes |
| N–D/C=N | Stretching | 2301.38 | — | — | Amines/imine-containing compounds |
| C=O | Stretching | 1841.75 | 1705.13 | 1730.20 | Esters, ketones, carboxylic acids |
| C=C | Stretching | — | 1612.54 | 1602.97 | Aromatic rings, phenolic compounds |
| C=C/N–H | Stretching/Bending | — | 1541.18 | — | Aromatic compounds, amines |
| N–H | Deformation | 1454.88 | — | — | Amines |
| C–H | Bending | 1343.64 | 1450.52 | 1450.02 | Alkanes, aromatic compounds |
| C–N | Stretching | — | 1344.43 | — | Amines |
| C–C/C–O | Stretching | 1226.11 | — | — | Esters, ethers |
| C–O | Stretching | — | 1240.27 | 1238.38 | Phenols, alcohols, esters |
| C–O | Stretching | — | 1201.69 | — | Ethers, alcohols |
| C–O | Stretching | — | 1087.89 | — | Secondary alcohols |
| C–O | Stretching | — | 1026.16 | 1026.16 | Primary alcohols, glycosides |
| Aromatic C–H | Out-of-plane bending | — | 868.00 | 860.28 | Aromatic substitution |
| Aromatic C–H | Bending | 761.63 | 790.84, 759.98, 732.97, 702.11 | 744.35 | Aromatic compounds |
| C–H | Wagging | 664.11 | — | — | Alkenes |
| C–OH | Bending | 394.14 | — | — | Phenolic compounds |
| C–Cl/C–Br | Stretching | — | 588–439 | — | Halogenated compounds |

***GC–MS Characterization of Ethanolic Leaf Extracts***

GC–MS analysis revealed distinct chemical profiles among the ethanolic extracts (Table 4). Fifteen compounds were identified in *A. indica*, eight in *P. guajava*, and ten in *O. gratissimum*. The predominant constituents differed among the three species. *A. indica* was characterized mainly by linoleic acid chloride (11.40%), an oxirane derivative (9.85%), and 2-ethyl-1-decanol (6.05%). In *P. guajava*, the major constituents were a heptadecane derivative (34.12%), 9, 12-octadecadienoyl chloride (20.06%), sulfurous acid ester (12.87%), and tetracontane (13.25%). The principal compounds identified in *O. gratissimum* were 9,12-octadecadienyloxy ethanol (26.14%), eicosyne (19.64%), oxacyclotetradecan-2,11-dione (12.54%), 1-heptanol, 6-methyl (10.70%), and methyl octadecanoate (10.39%).

The GC–MS chromatograms of the three ethanolic extracts are presented in Figure 1, while the major identified constituents and their reported biological activities are summarized in Table 4.

The GC–MS chromatograms are presented in Figures 1–3, while the major compounds identified in the ethanolic extracts are summarized in Table 4.

**Table 4: Major GC-MS Constituents (≥5% Peak Area) Identified in the Ethanolic Extracts**

| Plant | Compound | Peak Area (%) | Reported Activity |
|---|---|---|---|
| *A. indica* | Linoleic acid chloride | 11.40 | Antimicrobial, anti-inflammatory |
| *A. indica* | Oxirane derivative | 9.85 | Bioactive lipid |
| *A. indica* | 2-Ethyl-1-decanol | 6.05 | — |
| *P. guajava* | Heptadecane derivative | 34.12 | Antioxidant |
| *P. guajava* | 9,12-Octadecadienoyl chloride | 20.06 | Antimicrobial |
| *P. guajava* | Sulfurous acid ester | 12.87 | Antimicrobial |
| *P. guajava* | Tetracontane | 13.25 | Antimicrobial |
| *O. gratissimum* | 9,12-Octadecadienyloxy ethanol | 26.14 | Antimicrobial |
| *O. gratissimum* | Eicosyne | 19.64 | Antimicrobial |
| *O. gratissimum* | Oxacyclotetradecan-2,11-dione | 12.54 | Antioxidant-associated |
| *O. gratissimum* | 6-Octadecanoic acid methyl ester | 10.39 | Antimicrobial |
| *O. gratissimum* | 1-Heptanol, 6-methyl | 10.70 | Antimicrobial |

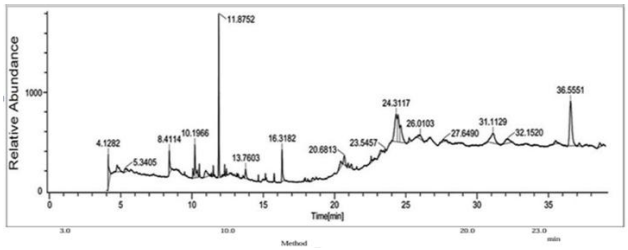


Figure 1: GCMS Chromatogram of *A. indica*

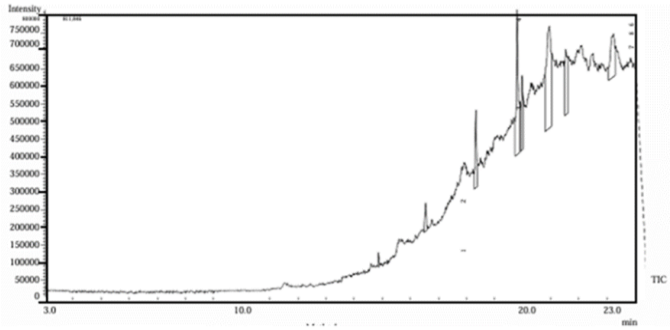


Figure 2: GCMS Chromatogram of *Psidum guajava* Ethanol Extract

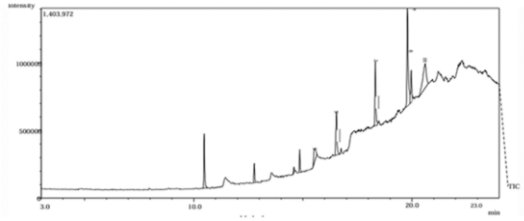


Figure 3: GCMS Chromatogram of *Ocimum gratissimum* Ethanol Extract

**Discussion**

The extraction solvent had a clear influence on both the quantity and composition of the leaf extracts obtained from *Azadirachta indica*, *Psidium guajava*, and *Ocimum gratissimum*. Ethanol consistently produced higher extraction yields than water, suggesting that it was more effective at recovering the bioactive constituents present in the leaves. This is not surprising because ethanol dissolves a wider range of phytochemicals, including both polar and moderately non-polar compounds, whereas water mainly extracts highly polar constituents. Similar observations have been reported in previous studies, where ethanol was found to be one of the most efficient solvents for extracting phytochemicals from medicinal plants (Altemimi et al., 2023; Martins et al., 2023; Plaskova & Mlček, 2023). Although *P. guajava* produced the highest extraction yield, differences among the three species are likely related to variations in their inherent chemical composition and metabolic characteristics rather than differences in the extraction procedure, since all samples were processed under identical condition. Ethanol can dissolve a broader range of plant constituents because of its intermediate polarity and ability to penetrate plant tissues, which may enhance the release of intracellular compounds compared with water.

The higher yield obtained from *P. guajava* has also been discussed. Differences in yield among the plants may be related to variations in cell-wall structure, moisture content, tissue characteristics, and the type and amount of compounds present in the plant material. These factors may explain the relatively higher amount of extractable constituents obtained from *P. guajava*.

Phytochemical screening further showed that all three plants are rich sources of secondary metabolites, although the abundance of individual compounds differed among species and extraction solvents. Phenols, flavonoids, tannins, alkaloids, saponins, and terpenoids were detected in all extracts, confirming the medicinal value traditionally attributed to these plants. The comparatively high phenolic and flavonoid contents of the ethanolic extract of *O. gratissimum*, together with the high tannin content of *P. guajava* and the elevated alkaloid concentration in *A. indica*, indicate that each species possesses a distinctive phytochemical profile (Akinjogunla and Itah 2024). These differences are expected because the biosynthesis of secondary metabolites is influenced by genetic factors, environmental conditions, and plant physiology (Altemimi et al., 2023; Mehra, 2023). Previous studies have similarly reported high concentrations of phenolic compounds and flavonoids in *O. gratissimum*, tannins in *P. guajava*, and diverse bioactive constituents in *A. indica*, supporting the findings of the present study (Beltrán-Noboa et al., 2023; Gutierrez-Montiel et al., 2023; Lepe de Alba et al., 2023).

The FTIR spectra supported the phytochemical results by confirming the presence of functional groups commonly associated with plant secondary metabolites. The prominent O–H, C=O, C=C, and C–O absorption bands observed across the ethanolic extracts indicate the occurrence of phenolic compounds, alcohols, esters, and aromatic compounds. Although slight differences in peak positions and intensities were observed among the three species, the overall spectral patterns were similar, reflecting the presence of related classes of phytochemicals. These observations agree with previous FTIR studies of medicinal plants, where similar absorption bands have been linked to phenolics, flavonoids, terpenoids, and other oxygenated compounds (Beltrán-Noboa et al., 2023; Tian et al., 2024). The consistency between the FTIR spectra and the phytochemical screening strengthens confidence in the chemical composition of the extracts.

The GC–MS analysis provided a more detailed picture of the chemical constituents present in the ethanolic extracts. Although the dominant compounds differed among the three plants, fatty acids, fatty acid derivatives, esters, long-chain hydrocarbons, and alcohols were identified in all extracts. Many of these compounds have previously been associated with antimicrobial, antioxidant, and anti-inflammatory activities, providing a chemical basis for the medicinal uses of these plants (Gutierrez-Montiel et al., 2023; Liu et al., 2024). The differences observed in the GC–MS profiles further indicate that each species contributes a unique combination of bioactive constituents, which may explain the variation in their reported biological activities.

An important observation from this study is the complementary information obtained from the three analytical techniques. Qualitative and quantitative phytochemical analyses identified the major classes of secondary metabolites present in the extracts; FTIR confirmed the functional groups associated with these compounds, while GC–MS enabled the identification of individual chemical constituents. When used together, these techniques provide a more complete understanding of the chemical composition of medicinal plants than any single analytical method. Such integrated characterization is valuable for the standardization of herbal materials and provides a scientific basis for future studies aimed at isolating bioactive compounds and evaluating their pharmacological properties (Lepe de Alba et al., 2023; Tian et al., 2024).

Overall, the findings demonstrate that ethanol is a more effective extraction solvent than water for recovering phytochemically rich extracts from *A. indica*, *P. guajava*, and *O. gratissimum*. The diversity of secondary metabolites identified by phytochemical screening, FTIR, and GC–MS analyses supports the long-standing medicinal use of these plants and highlights their potential as sources of natural compounds for pharmaceutical development. Further studies should focus on isolating the major bioactive constituents and investigating their biological activities, mechanisms of action, and possible synergistic interactions.

**CONCLUSION**

This study showed clear differences in the phytochemical composition, extraction yield, and antibacterial activity of *Azadirachta indica*, *Psidium guajava*, and *Ocimum gratissimum* extracts. Ethanol generally gave higher yields than water, with ethanolic *P. guajava* producing the highest yield (11.28%). The ethanolic *O. gratissimum* extract had the highest phenolic content, while the aqueous *A. indica* extract contained more alkaloids than the ethanolic extract, showing that the choice of solvent can influence the recovery of specific phytochemicals. Antibacterial activity also varied among the extracts and bacterial isolates tested. Overall, ethanolic *P. guajava* was most effective for extract recovery, while *O. gratissimum* was particularly rich in phenolic compounds. These findings provide useful guidance for selecting plant species and extraction solvents for further development of plant-based antimicrobial preparations.

This study provides a comprehensive comparative characterization of the leaf extracts of *Azadirachta indica*, *Psidium guajava*, and *Ocimum gratissimum* using phytochemical screening, FTIR spectroscopy, and GC–MS analysis. The findings showed that the three medicinal plants possess diverse chemical constituents, although their phytochemical profiles differed in composition and abundance. These differences suggest that each species offers

distinct bioactive compounds that may contribute to their reported medicinal properties.

The combined analytical approach adopted in this study generated valuable baseline information for the identification, quality assessment, and standardization of these medicinal plants. The results also strengthen existing evidence supporting their potential as sources of natural products for pharmaceutical and other biomedical applications. Further studies are recommended to isolate the major bioactive constituents, investigate their mechanisms of action, and evaluate their therapeutic potential through in vitro and in vivo studies.

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**COMPETING INTERESTS**

Authors have declared that no competing interests exist

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