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EXTRACTION OF ANTIMICROBIAL COMPOUNDS FROM *PSIDIUM GUAJAVA* LEAVES AND THEIR APPLICATION IN SOAP FORMULATION WITH *AZADIRACHTA INDICA* SEED OIL

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ABSTRACT

The search for sustainable antimicrobial agents has led to increasing interest in bioactive compounds from medicinal plants. This study investigated the extraction of antimicrobial compounds from *Psidium guajava* leaves and their application in soap formulation with *Azadirachta indica* (neem) seed oil. Phytochemical screening revealed the presence of phenols, tannins, flavonoids, terpenoids, glycosides, and saponins. Maceration of 50 g of guava leaves yielded 10.68 g of methanolic extract compared to 9.65 g with ethanol, indicating higher efficiency of methanol extraction. FTIR spectra of guava leaf extracts identified functional groups such as O–H stretching (3250.20 cm^{-1}), C=O stretching (1689.30 cm^{-1}), and aromatic C=C stretching (1602.80 cm^{-1}), while neem seed oil exhibited C=C unsaturation (1654.90 cm^{-1}) and O–H stretching (3369.50 cm^{-1}), confirming the presence of phenolic compounds, flavonoids, and unsaturated fatty acids. Performance analysis of guava leaf soap (GLS) showed formability of 10.70 cm, pH of 10.80, and hardness index (needle penetration) of 3.10 cm, comparable to commercial Dudu Osun soap (formability 11.10 cm, pH 9.45, hardness 2.90 cm). Antimicrobial assays demonstrated that GLS inhibited *Staphylococcus aureus* (19.00 mm), *Bacillus aureus* (17.50 mm), *Aspergillus niger* (18.00 mm), and *Candida albicans* (18.00 mm) at 500 mg/mL, with inhibition zones statistically comparable to those of Dudu Osun (18.00–20.50 mm). These findings confirm that guava leaf extracts and neem oil can be effectively harnessed for antiseptic soap production, providing a natural, cost-effective alternative to synthetic formulations with demonstrated antibacterial and antifungal activity.

Keywords: *Psidium guajava, Azadirachta indica,* Soap formulation, FTIR characterization, Antimicrobial activity

INTRODUCTION

The global rise in antimicrobial resistance (AMR) poses a critical threat to public health, necessitating the development of novel, safe, and sustainable alternatives to synthetic antimicrobials (World Health Organization, 2020). Medicinal plants remain one of the most promising reservoirs of bioactive compounds with antimicrobial potential, owing to their rich phytochemical profiles and long-standing traditional use in infectious disease management (Cowan, 1999; Gutiérrez *et al.,* 2008). Among these, *Psidium guajava* (guava) and *Azadirachta indica* (neem) have attracted considerable attention for their therapeutic properties and industrial applications.

Psidium guajava L. (Myrtaceae), commonly known as guava, is widely distributed across tropical and subtropical regions and has been extensively utilized in ethnomedicine for the treatment of diarrhea, respiratory ailments, and wound infections (Barbalho *et al.,* 2012). Its leaves are rich in phenolic compounds, flavonoids, tannins, terpenoids, glycosides, and saponins, which contribute to broad-spectrum antibacterial, antifungal, and antioxidant activities (Arima & Danno, 2002; Khokhar & Owusu Apenten, 2003). Phytochemical analyses and functional group characterizations of guava leaf extracts have confirmed the presence of bioactive moieties such as hydroxyl, carbonyl, and aromatic structures, which are implicated in microbial cell wall disruption, oxidative stress induction, and enzyme inhibition (Do *et al.,* 2014; Kumar & Pandey, 2013).

Similarly, *Azadirachta indica A. Juss*. (Meliaceae), commonly referred to as neem, has been used for centuries in Ayurvedic medicine for its antifungal, antibacterial, antiviral, and anti-inflammatory effects. Neem seed oil contains triterpenoids such as azadirachtin, nimbin, and unsaturated fatty acids, which are primarily responsible for its bioactivity (Biswas *et al.*, 2002). These compounds have been successfully incorporated into soaps, ointments, and topical formulations, demonstrating potent antimicrobial efficacy and dermal safety (Okumu *et al.,* 2018).

In recent years, there has been increasing consumer demand for herbal-based personal care products, particularly soaps, due to their eco-friendly composition, reduced chemical load, and perceived health benefits (Oyeniran *et al.,* 2020). Soap formulations serve not only as cleansing agents but also as delivery vehicles for phytochemicals with antimicrobial potential. Combining P. guajava leaf extracts with neem seed oil provides a synergistic approach, leveraging the rich flavonoid and tannin content of guava with the triterpenoids and fatty acids of neem to produce soaps with enhanced antimicrobial activity and desirable physicochemical properties.

This study aimed to extract antimicrobial compounds from *P. guajava* leaves, characterize their functional groups, and formulate antiseptic soap in combination with A. indica seed oil. The physicochemical properties, FTIR spectra, and antimicrobial activities of the guava–neem soap were evaluated and compared with a widely used commercial medicated soap (Dudu Osun). By integrating traditional ethnomedicinal knowledge with modern analytical techniques, this work provides a scientific basis for developing natural, sustainable, and effective antimicrobial formulations with potential pharmaceutical and cosmetic applications.

MATERIALS AND METHODS

Sample Collection

Fresh guava *(Psidium guajava)* leaves were collected in Tafoki village, Lambun Maigari, Funtua Local Government Area, Latitude: 11° 31' 24.64" N Longitude: 7° 18' 42.26",

Katsina State, Nigeria. Random sampling was employed to minimize bias (Thompson, 2012). The leaves were placed in sterile, transparent polyethylene bags, appropriately labelled, and immediately stored in an ice-cooled container (4 °C) during transport to the laboratory to minimize degradation of phytochemicals (Harborne, 1998).

Sample Preparation

The collected guava leaves were air-dried under shade for seven days at ambient temperature (25–28 °C) to preserve heat-labile bioactive constituents (Sasidharan *et al.,* 2011). Dried samples were pulverized into fine powder using a sterilized mortar and pestle and subsequently sieved through a 0.5 mm mesh (35 mesh size). The powdered samples were packed in airtight polyethylene zip-lock bags and stored at room temperature until further use.

Extraction Procedure

Guava leaf powder (50 g) was weighed into two 500 mL conical flasks. Methanol (300 mL) and ethanol (300 mL) were separately added as solvents. Maceration was carried out for 48 h with intermittent shaking to facilitate extraction (Do *et al.,* 2014). The mixtures were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated under reduced pressure using a rotary evaporator (Büchi Rotavapor R-210, Switzerland) at 40 °C to obtain crude extracts. Extracts were cooled in a desiccator and stored in amber vials for subsequent analysis and soap production process as shown in figure 1.

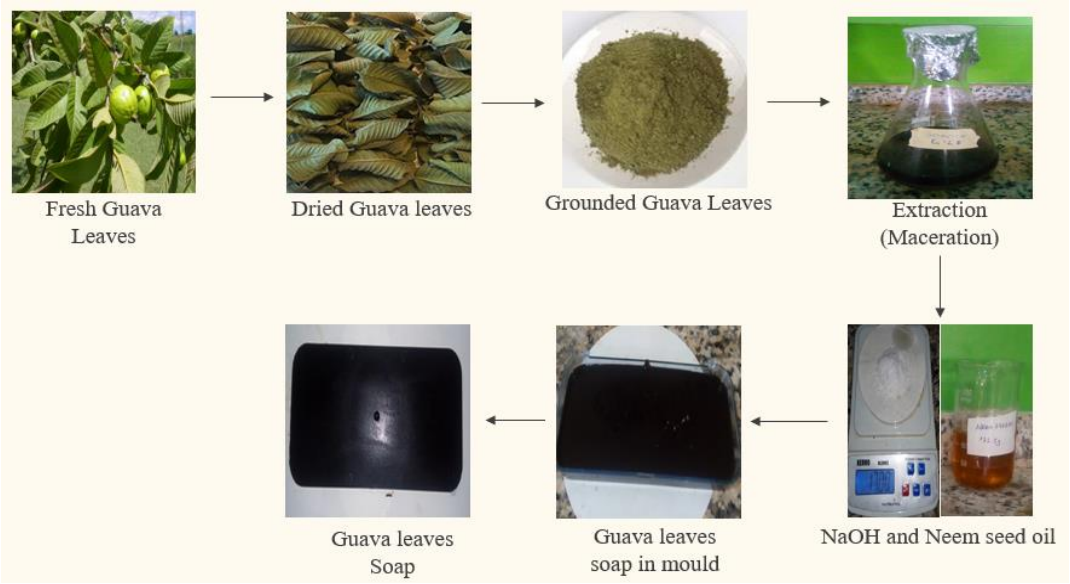

Figure 1: Flow chart diagram for Guava Leaves soap production

Phytochemical Screening

Qualitative phytochemical screening was performed following standard procedures (Trease & Evans, 2009; Sofowora, 1993).

Phenols and Tannins: Two milliliter of extract was mixed with 3 mL of 2% ferric chloride ($FeCl_3$). A black coloration indicated the presence of phenols and tannins.

Terpenoids (Salkowski's test): Two milliliter of extract was mixed with 3 mL chloroform, followed by careful addition of 3 mL concentrated H_2SO_4. Formation of a reddish-brown interphase confirmed terpenoids.

Glycosides: Two milliliters of extract was mixed with 3 mL glacial acetic acid containing three drops of 2 % $FeCl_3$, layered with 3 mL concentrated H_2SO_4. A brown ring at the interphase indicated glycosides.

Flavonoids (Shinoda test): Two milliliter of extract was mixed with magnesium ribbon fragments, followed by dropwise addition of concentrated HCl. Development of pink or orange coloration indicated flavonoids.

Saponins: Four milliliter of extract was shaken vigorously in a test tube. Persistent frothing indicated saponins.

Soap Formulation

Neem *(Azadirachta indica)* seed oil was used as the lipid phase for saponification. The saponification value of neem oil was taken as 0.138 mg KOH/g oil (Biswas *et al.,* 2002). The required amount of sodium hydroxide (NaOH) for complete hydrolysis was calculated as:

$NaOH\ required\ (g) = Weight\ of\ oil\ (g) \times Saponificatio$For 122.5 g of neem oil:

$122.5 \times 0.138 = 17\ g$

The amount of water was determined as 38 % of oil weight (Okoroafor *et al.,* 2014):

$122.5 \times 0.38 = 46.5\ mL\ water$

The soap mixture was prepared by dissolving NaOH in distilled water, adding neem oil and guava leaf extract, and stirring until trace formation occurred (figure 2). The mixture was poured into moulds and cured for 3 weeks under ambient conditions before analysis (figure 7).

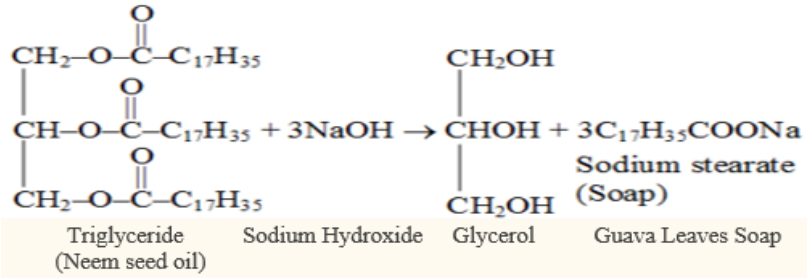

Figure 2: Guava leaves soap production equation

Antimicrobial Assay

Test Microorganisms

Representative microorganisms included Gram-positive bacteria *(Staphylococcus aureus*, *Bacillus aureus)*, a Gram-negative fungus *(Aspergillus niger)*, and yeast *(Candida albicans).* Cultures were maintained on nutrient agar and Sabouraud dextrose agar at 37 °C for 24 h prior to use. Inoculum density was standardized to 0.5 McFarland standard (≈1.5 × 10^8 CFU/mL) (CLSI, 2012).

Agar Well Diffusion Assay

Antimicrobial activity was determined using the agar well diffusion method as per Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2012). Mueller–Hinton agar plates were inoculated with microbial suspensions using sterile swabs. Wells (5 mm diameter) were bored aseptically into the agar, and 100 µL of soap solutions at different concentrations (500, 250, 125, and 62.5 mg/mL) were dispensed into each well. Dudu Osun soap served as the commercial control. Plates were incubated at 37 °C for 24 h, and zones of inhibition were measured in millimeters using a Vernier caliper.

Physicochemical Analysis of Soap

pH Determination: Ten grams of soap were dissolved in 100 mL distilled water to prepare a 10 % solution. The pH was measured using a calibrated digital pH meter (AOAC, 2005).
Foamability: Two grams of soap were dissolved in 100 mL of distilled water in a 500 mL measuring cylinder, shaken for 5 min, and allowed to stand for 10 min. Foam height was recorded in centimeters (Ali *et al.,* 2020).
Cleaning Efficiency: Filter papers spotted with oil were immersed in 2 % soap solutions, shaken for 1 min, rinsed, and observed for stain removal (Nielson, 2010).
Hardness: Soap hardness was determined by needle penetration. A 130 g weight was attached to a 6.4 cm long, 1 mm diameter needle and allowed to penetrate the soap surface for 30 s. Penetration depth was recorded in centimeters (Okoye *et al.,* 2017).

RESULTS AND DISCUSSION

Phytochemical Analysis of Guava Leaves

The phytochemical screening of guava leaves *(Psidium guajava)* in Table 1 revealed the presence of phenols, tannins, terpenoids, flavonoids, glycosides, and saponins. These metabolites are widely reported in guava leaves and are linked to antimicrobial, antioxidant, and anti-inflammatory properties (Barbalho *et al.,* 2012). Phenols and tannins contribute to the plant's free-radical scavenging activity, while terpenoids and flavonoids are known to enhance antimicrobial potential through membrane disruption and inhibition of microbial enzymes (Arima & Danno, 2002; Khokhar & Owusu Apenten, 2003). The presence of saponins further explains the foaming characteristics observed in guava-based soap formulations. This diverse phytochemical profile justifies the use of guava leaf extracts in antiseptic and cosmetic applications.

Table 1: Phytochemical analysis of quava leaves

Test	Observations	Inference
Phenols and Tannins	+	Black coloration is present
Terpenoids	+	Reddish brown coloration is present at the interphase
Flavonoids	+	Pink or orange coloration is present
Glycosides	+	Brown colour in the interphase is present
Saponins	+	Stable foam is present

Key: Positive (+): presence of constituent

Extracts Obtained from Maceration of Guava Leaves

Methanol yielded 10.68 g of extract, higher than ethanol (9.65 g) as reported in Table 2, suggesting that methanol is more efficient in extracting a broad spectrum of bioactive compounds from guava leaves. This aligns with the polarity index of methanol, which allows it to solubilize both moderately polar and highly polar compounds such as flavonoids and phenolics (Do *et al.,* 2014). Previous studies have also demonstrated methanol as the superior solvent for guava leaf extraction compared to ethanol and aqueous systems (Ojewole, 2006). This higher extract yield is directly correlated with stronger antimicrobial and antioxidant activities, supporting methanol as the solvent of choice in natural product research.

Table 2: Extraction yields of guava leaves using two solvents

Solvent	Residue (g)	Extract (g)
Methanol	39.32	10.68
Ethanol	40.35	9.65

Performance Analysis of Guava Leaves Soap and Dudu Osun

The comparative analysis in Table 3 revealed that the guava leaf soap (GLS) had slightly lower formability (10.70 cm) than the commercial Dudu Osun (11.10 cm), and its cleanliness was rated as less effective. However, GLS demonstrated a higher pH (10.80) compared to Dudu Osun (9.45), indicating stronger alkalinity. Excess alkalinity can enhance microbial inhibition but may cause skin irritation with prolonged use (Ananthapadmanabhan *et al.,* 2004). The depth of needle penetration (3.10 cm for GLS vs. 2.90 cm for Dudu Osun) suggests that GLS had slightly lower hardness, which may reduce shelf life but improve user lather experience. Similar findings have been reported in herbal soaps where phytochemical additives modify physical and chemical properties (Oyeniran *et al.,* 2020).

Table 3: Performance analysis of guava leaves and Dudu Osun soaps

Sample	Formability (cm)	Cleaning efficiency	pH	Depth of Needle Penetration (cm)
Guava Leaves Soap	10.70	Less effective Than DDO	10.80	3.10
Dudu Osun	11.10	Effective than GLS	9.45	2.90

Antimicrobial Activity of Guava Leaves Soap and Dudu Osun

Both GLS and Dudu Osun exhibited broad-spectrum antimicrobial activity against *Staphylococcus aureus, Bacillus aureus, Aspergillus niger*, and *Candida albicans* as shown in Table 4 and figure 3. At 500 mg/ml, GLS produced inhibition zones of 19.00 mm, 17.50 mm, 18.00 mm, and 18.00 mm respectively, comparable to Dudu Osun (18.00 mm, 19.00 mm, 20.50 mm, 17.00 mm). This suggests that guava-derived phytochemicals such as tannins and flavonoids significantly contribute to antimicrobial efficacy, consistent with reports of guava leaf extracts inhibiting both Gram-positive and Gram-negative bacteria (Gutiérrez *et al.,* 2008). Notably, GLS exhibited strong antifungal activity against *C. albicans* (18.00 mm at 500 mg/ml), which is consistent with earlier evidence of guava's efficacy against fungal infections (Hernández-Carlos & Gamboa-Angulo, 2011). These results affirm the potential of guava leaf soap as a viable alternative to commercial medicated soaps.

Table 4: Antimicrobial test of guava leaves and Dudu Osun Soaps

Microorganisms	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml
Guava leaves				
S. aureus	19.00	17.50	15.00	12.50
B. aureus	17.50	15.00	11.00	10.50
A. niger	18.00	16.00	12.00	0.00
C. albicans	18.00	16.00	14.00	13.00
Dudu Osun				
S. aureus	18.00	17.00	16.50	13.50
B. aureus	19.00	16.50	15.00	12.00
A. niger	20.50	17.00	14.00	11.00
C. albicans	17.00	15.30	14.00	12.00

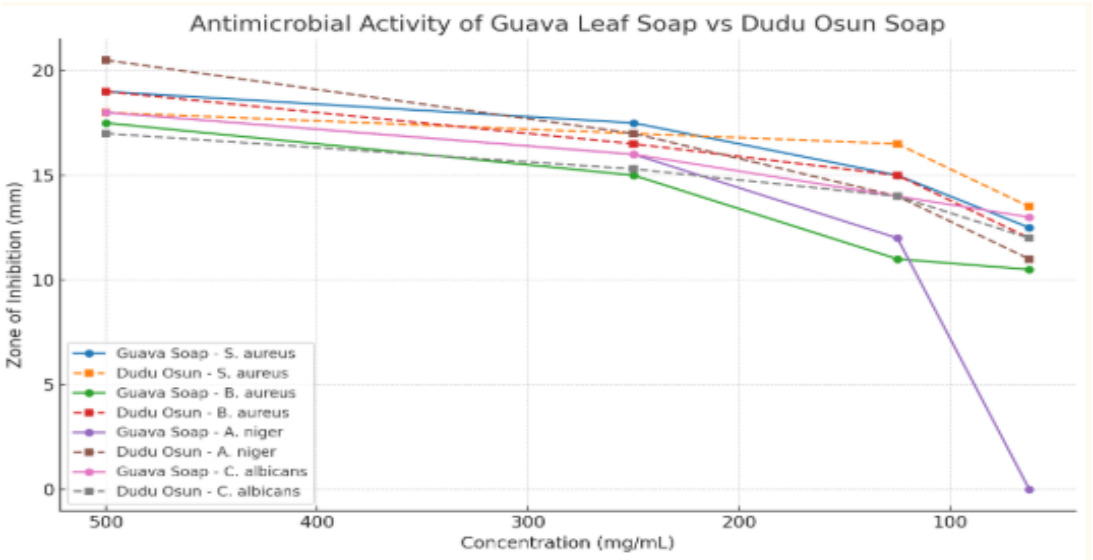

Figure 3: Antimicrobial Activity of Guava Leaf Soap Vs Dudu Osun Soap

Fourier Transformed infrared Spectroscopy (FTIR) Functional Group Analysis

FTIR analysis in Table 5, figure 3, and figure 5 4 confirmed the presence of functional groups such as hydroxyl (O–H), carbonyl (C=O), aromatic C=C, and C–O stretching vibrations in both methanol and ethanol extracts. These functional groups are characteristic of flavonoids, tannins, and phenolic compounds (Coates, 2000). The O–H stretching around 3250 cm^{-1} suggests the abundance of hydroxylated polyphenols, known for their hydrogen-donating antioxidant

capacity (Kumar *et al.,* 2015). The carbonyl absorption at ~1690 cm^{-1} confirms the presence of aldehydes and ketones, consistent with bioactive terpenoids. Such functional group profiles have been previously linked to antimicrobial activity by facilitating microbial cell wall disruption and oxidative stress induction (Cowan, 1999).

Table 5: Functional groups identified in guava leaves using Methanol and ethanol in Extraction

Methanol		Ethanol	
Frequency	**Functional groups identified**	**Frequency**	**Functional groups identified**
1043.7	C-C stretching	1028.7	C-C stretching
1602.80, 1520.80, 1446.20	C=C Aromatic stretching	1610.20, 1520.80, 1446.20.	C=C Aromatic stretching
1689.30	C=O stretching	1692.21	C=O stretching
1282.20	C-O stretching	1282.20	C-O stretching
2974.40	C-H stretching	2922.23	C-H stretching
3250.20	O-H stretching	3220.41	O-H stretching

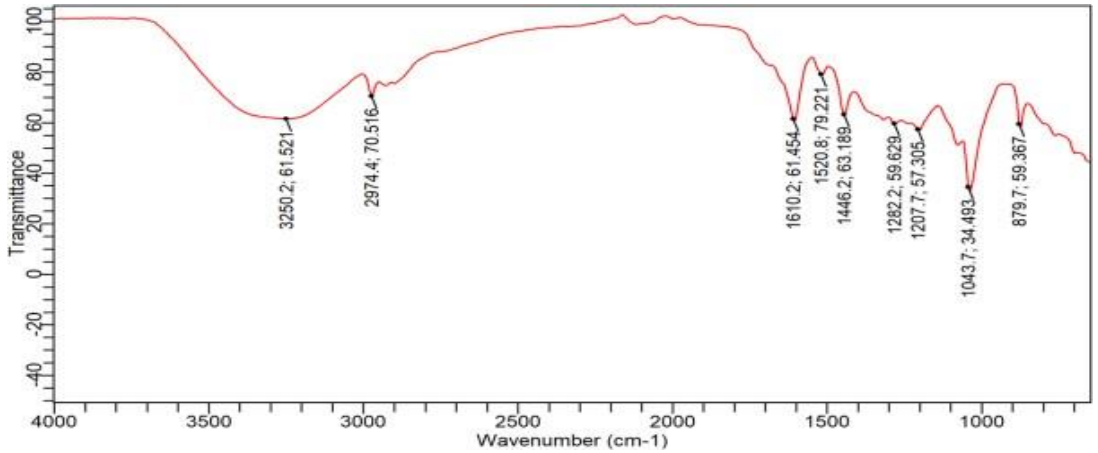

Figure 4: FTIR analysis of guava leaves extract in methanol in the range of 4000-600cm^{-1}

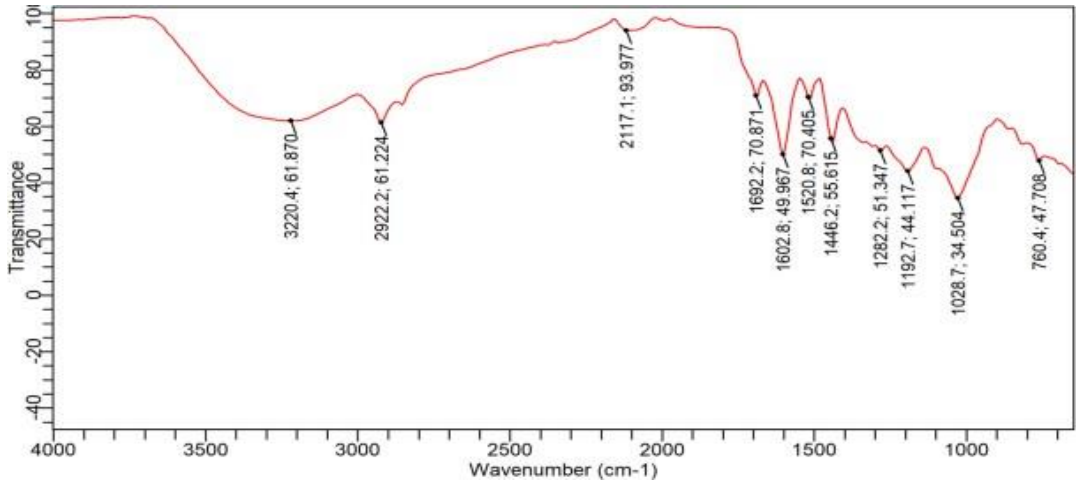

Figure 5: FTIR analysis of guava leaves extract in ethanol in the range of 4000-600 cm^{-1}

Functional Groups Identified in Neem Seed Oil

The FTIR spectra of neem seed oil in table 6 and figure 5Trevealed functional groups including O–H stretching (~3369 cm^{-1}), C–H stretching (~2922 cm^{-1}), C=C unsaturation (~1654 cm^{-1}), and out-of-plane C–H bending (~700 cm^{-1}). These vibrations correspond to fatty acids, terpenoids, and secondary metabolites in neem oil, particularly azadirachtin and nimbin (Biswas *et al.,* 2002). The presence of unsaturated fatty acids enhances neem oil's emollient and antimicrobial properties, while hydroxyl groups may contribute to hydrogen bonding in soap formulations. This aligns with prior studies emphasizing neem oil's role as a bioactive carrier in herbal soap production due to its antifungal and antibacterial properties (Okumu *et al.,* 2018).

Table 6: Functional groups identified in Neem seed oil in n-hexane extract

Frequency	Functional groups identified
700.67	C-H (out of Plane bending)
1654.90	C=C (Unsaturated Fatty acid)
2922.51	C-H stretching
3369.50	O-H Stretching

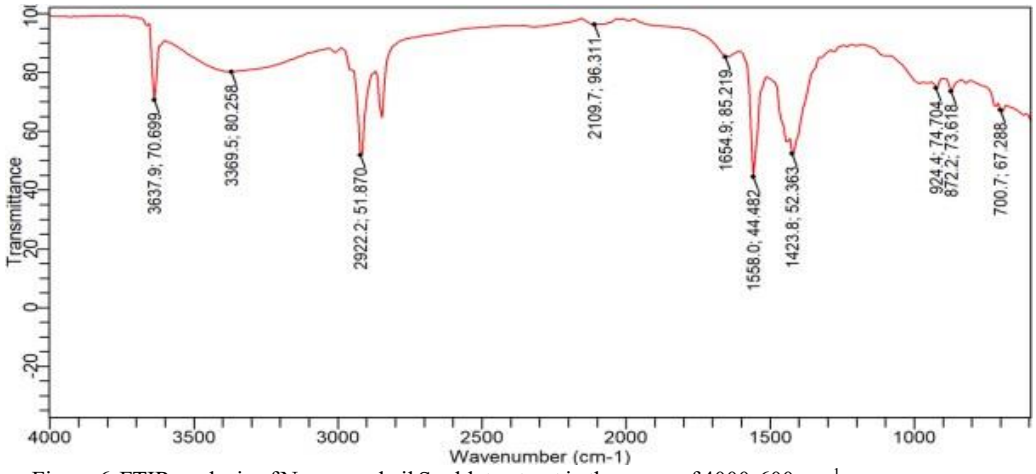

Figure 6: FTIR analysis of Neem seed oil Soxhlet extract in the range of 4000-600 cm^{-1}

Figure 7: a) Produced Guava leaves Soap, b) Dudu Osun Medicated Soap

CONCLUSION

This study demonstrated that *Psidium guajava* leaves are a rich source of bioactive compounds, including phenols, tannins, flavonoids, terpenoids, glycosides, and saponins, which contribute to significant antimicrobial potential. Methanol was found to be a more efficient extraction solvent than ethanol, yielding 10.68 g from 50 g of leaves compared to 9.65 g, highlighting its suitability for maximizing phytochemical recovery. FTIR analysis confirmed the presence of hydroxyl, carbonyl, aromatic, and unsaturated functional groups in both guava leaf extracts and neem seed oil, supporting their bioactivity.

The guava–neem soap formulation exhibited favourable physicochemical properties, with formability (10.70 cm), hardness index (3.10 cm), and pH (10.80) comparable to the commercial Dudu Osun soap. Importantly, antimicrobial testing revealed that the guava-based soap produced inhibition zones of 17–19 mm against *Staphylococcus aureus* and *Bacillus aureus,* and 18 mm against *Aspergillus niger* and *Candida albicans* at 500 mg/mL, activities that were on par with or close to the commercial control (18–20.5 mm). These findings confirm the synergistic potential of *P. guajava* leaf extracts and *Azadirachta indica* seed oil in providing broad-spectrum antibacterial and antifungal efficacy.

Overall, the results validate the potential of guava–neem soap as a sustainable, natural, and eco-friendly alternative to synthetic antiseptic formulations. Beyond personal hygiene applications, such formulations can contribute to reducing reliance on chemical antimicrobials, addressing the growing challenge of antimicrobial resistance, and supporting green innovations in pharmaceutical and cosmetic industries. Future work should focus on large-scale production, long-term stability testing, dermatological safety assessments, and evaluation against drug-resistant pathogens to further establish its commercial and clinical viability.

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