INMRJ
International Nexus Multidisciplinary Research Journal
https://inmrj.org
Impact Factor: 5.0 **- Vol:** 3 **Issue:2,** 2026

ANTIFUNGAL EFFICACY OF *Psidium guajava* (GUAVA) AND *Citrus sinensis* (ORANGE) LEAVES EXTRACT AGAINST *Candida albicans.*

Nwachukwu, I.O
Department of Microbiology,
Faculty of Microbiology
Imo State University, Owerri
Corresponding email: ogechinwachukwu2081@gmail.com

ABSTRACT

This study evaluated the antimicrobial efficacy of *Psidium guajava* (Guava) and *Citrus sinensis* (Orange) leaf extracts against *Candida albicans*. The practical research was conducted at the microbiology laboratory of Imo State University, using plant materials sourced from the Federal Polytechnic Nekede, Owerri. The leaves were processed into fine particles and subjected to aqueous and ethanolic extraction via maceration. Phytochemical screening revealed the presence of flavonoids, tannins, saponins, phenols, and alkaloids in guava extracts, with ethanol extract showing stronger concentrations of flavonoids, tannins, and phenols. Orange extracts contained fewer phytochemicals, with alkaloids absent in both ethanol and aqueous forms. Antifungal activity assessed by agar well diffusion demonstrated dose-dependent inhibition, with guava ethanol extract producing the largest zone of inhibition (17.0 ± 0.5 mm at 100 mg/mL), followed by orange ethanol extract (10.0 ± 0.6 mm), guava aqueous extract (11.0 ± 0.8 mm), and orange aqueous extract (8.5 ± 0.4 mm). Fluconazole, the positive control, exhibited the highest activity (22.0 ± 0.3 mm). Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) assays confirmed fungicidal activity for all extracts (MFC/MIC ratio = 2), with guava ethanol extract being the most potent (MIC 12.5 mg/mL, MFC 25 mg/mL). Statistical analysis (ANOVA) showed significant differences among extracts ($P < 0.05$), with guava ethanol extract outperforming orange extracts. Overall, ethanol extracts exhibited stronger antifungal activity than aqueous extracts, and guava demonstrated greater efficacy than orange. These findings suggest that guava leaves, particularly ethanol extracts, possess promising antifungal potential attributable to their rich phytochemical content, and may serve as a natural alternative in managing *Candida albicans* infections.

Key words; *Psidium guajava, Citrus sinensis, Candida albicans*, Antifungal activity, Leaf extract, Minimum Inhibitory Concentration (MIC), Minimum Fungicidal Concentration (MFC).

INTRODUCTION

Plants are among the most prominent candidates in herbal medicine (Abduallah, *et al*., 2024). Products of plants are easily available, cheap, and have the slightest tendency to develop resistance, due to which they could be proven beneficial for health (Ahmad *et al*., 2023). Herbal medicines are broadly used by the lower to middle classes to promote, prevent, and rehabilitate (Dalal *et al*., 2023). Plants have also been used as an alternative to antiparasitic drugs due to their pharmacological characteristics described in the recent past (Hussain *et al*., 2024). Knowledge about the usage of therapeutic plants is a custom that is continued from one generation to another. Level of education, ecological zone, and ethnicity are aspects that are associated with the knowledge and usage of medicinal plants.

According to the World Health Organization (WHO), 60% of the population globally, and a whopping 80% in developing countries depend on medicinal plants (Khan and Ahmad 2018). Medicinal plants produce a variety of metabolites that show important biological activity and, are, therefore useful for a wide range of conditions (Ali, *et al*., 2019). Metabolites such as phenols, flavonoids, alkaloids, tannins, and saponins are found in the plants' leaves, roots, and stems. Plant metabolites have several beneficial properties, such as anti-inflammatory, antihypertensive, anti-ageing, antibacterial, antifungal, antiviral, anti-cancer, antioxidants, neuroprotective, and anticoagulant effects (Lesellier *et al*., 2021). For instance, the metabolite flavonoid is associated with antioxidant activity and saponins are associated with antimicrobial activity (Cankaya and Somuncuoglu, 2021). There has been an increasing interest in plants and their medicinal value over the last few decades.

Guava plant (*Psidium guajava*) Linn. belonging to family Myrtaceae, a traditionally used plant because of its food and nutrition value. Guava is widely grown in tropical and many areas like India, Bangladesh, Florida, and West Indies. Different parts of the *Psidium guajava* are reported to be used in folk medicine. Various parts of the plant like root, bark, leaves and fruits are found to possess many pharmacological properties as it is used in the treatment of various disorders (Abubakar *et al*., 2022). Various evidences depict that the leaves and bark of *P. guajava* tree possess a long history of medicinal uses. The aqueous extract of guava leaves has been reported to be efficacious in the treatment of various types of gastrointestinal disturbances such as diarrhea, inhibition of the peristaltic reflex and gastroenteritis (Ibeh *et al*., 2021). Moreover, the whole plant is used as skin tonic and is employed in the treatment of female related disease like dysmenorrhoea, miscarriages, uterine bleeding and premature labor (Mansurat *et al.,* 2023). Recent studies on the pharmacological properties of the bark, fruit and leaves depicts antibacterial, hypoglycaemic, anti-inflammatory, antipyretic, spasmolytic and central nervous system depressant activities. Bark tincture showed fungicidal activity at different concentrations but exhibit only fungistatic property in case of *Candida albicans*. Leaf extract of *Psidium guajava* also reported for the anti-bacterial activity on *Staphylococcus aureus* due to the protein degrading activity of the leaf extract (Biswas *et al*., 2013). The aqueous extract was more potent in inhibiting the growth of *E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* than the organic extracts. The Gram-negative bacteria were less susceptible to the effect of crude drugs.

The genus Citrus belongs to the family of Rutaceae and is native tropical and subtropical areas in Southeast Asia (Rahayu *et al.,* 2024). The citrus plants are grown worldwide and ranks top in production and trade among the fruit trees. Citrus fruits are richer sources of bioactive compounds having beneficial effect on human health such as vitamin C, carotenoids, flavonoids, limonoids, essential oils, acridone alkaloid, minerals and vitamin B complex. Majority of citrus fruits are eaten fresh such as sweet orange, mandarins, grapefruits etc.

Conventional drugs are expensive and the western health facilities are also inaccessible to rural people (WHO, 2010). Medicinal plants have been used since time immemorial to treat and prevent human ailments because they have components of therapeutic value. It is however noted that medicinal plants are readily available, they have little side effects and there is extensive local knowledge on herbal medicine amongst the communities (Awoke, *et al*., 2024). Some of the known good sources of pharmacologically active compounds are natural products from fungi and higher plants. Many of the effective drugs such as anti-malarial, anticancer, anti-diabetic and antibiotics such as atropine and ergometrine compounds have been purified from medicinal plants. Medicinal plants are also sources of many active ingredients in the pharmaceutical industries (Oliveira, *et al*., 2025). The popularity of plants medicine is increasing because of their biodegradability, least persistence and less toxic to non-target organisms, economic and easy availability. The aim of this study is to evaluate and compare the antifungal efficacy of *Psidium guajava* (Guava) and *Citrus sinensis* (Orange) leaf extracts against *Candida albicans* and assess their potential as alternative antifungal agents.

MATERIALS AND METHODS

Study Area

This study was carried out in the Microbiology Laboratory of Imo State University (IMSU), Owerri, Imo State, Nigeria. The university is located in Owerri, the capital city of Imo State, situated in the southeastern region of Nigeria. Owerri lies approximately between latitudes 5°29′N and 5°34′N and longitudes 7°02′E and 7°07′E.

The Microbiology Laboratory at IMSU is well-equipped with facilities for microbiological research, including instruments for microbial culture, identification, and biochemical analysis. The laboratory provides a controlled environment suitable for experimental work in microbiology, ensuring accuracy and reliability of results.

Imo State University serves as a major academic and research institution in the region, with the Microbiology Department playing a vital role in training students and conducting studies relevant to public health, environmental microbiology, and biotechnology. The choice of this laboratory as the study area was influenced by its accessibility, availability of standard equipment, and its relevance to the objectives of this research.

Collection of Plant Materials

Psidium guajava (Guava) and *Citrus sinensis* (Orange) leaves were collected from the botanical garden of Federal Polytechnic Nekede Owerri and they were identified by a Botanist from the Department of Environmental Biology at Federal Polytechnic Owerri, Imo State.

Processing of Plant Materials

The plant leaves were reduced in size with sterilized knife in order to facilitate drying and milling after which were dried at room temperature in order to prevent loss of active constituents which may be thermo-labile. Both leaves were separately grounded using sterile mortar and pestle and then milled to fine particles using a big miller, after which they were stored in air tight containers.

Sterilization of Equipment and Media

All glassware' used in this study, including petri dishes, conical flasks and pipettes were thoroughly washed and rinsed with running water and dried. They were subsequently wrapped in aluminum foil and sterilized in an oven. The culture media was sterilized by autoclaving at 121 ^{0} C for 15 minutes. The work surface was disinfected with 70% ethanol.

Extraction Of Plant Material (Maceration)

About 100 grams of powdered parts of *P. guajava* and *C sinensis* plant were soaked in 200 ml of ethanol and water contained in 500 ml conical flasks and covered with cotton wool separately. Thereafter, it was shaken occasionally for one week. The extracts were filtered using Whatman No. 1 filter paper to get a good filtrate. The filtrate was evaporated using an oven at 40 °C to obtain a dry extract. The dried extracts were stored at 4°C for further use.

The percentage yield of the plant extract obtained were calculated as Percentage Yield (%) = weight of plant sample after extraction weight of dried powder extract × 100.

Phytochemical Analysis

Test For Tannins

The plant extracts were mixed with 15% ferric chloride solution. The change in color was observed. The appearance of blue color showed the presence of tannin (Biwas *et al*., 2013).

Test For Alkaloids

The plant extracts were dispensed in 10 ml methanol in a test tube containing chloroform and the solution were removed with dilute H2SO4 and acid layer taken and tested for presence of alkaloids:

Dragendroff's test; About 5 ml of acid layer of test solution were mixed with 5 mL of Dragendroff's reagent (potassium bismuth iodide solution) in a test tube and shaken vigorously to obtain a stable solution. Thereafter, 5 mL of dilute HCl was added. The appearance of orange-red precipitate indicates the presence of alkaloids (Biwas *et al*., 2013).

Test for Cardiac Glycosides (Keller-Kilian's test)

About 0.5 mL of glacial acetic acid was dissolved in conical flask containing 50 ml of test solution and one drop of ferric chloride solution was added in the conical flask. Then 0.5ml of concentrated sulphuric acid were added to the test solution and reaction noted (Hussain, *et at.*, 2015).

Test For Saponins:

About 1 mL of the plant extract were dispensed in test tube containing water and then shaken occasionally. The sample were warmed and any appearance of frothing, which persist on warming will show preliminary evidence for the presence of saponins. After which, few drops of olive oil was added to 1ml of the extracts and vigorously shaken. Formation of soluble emulsion in the extract indicates the presence of saponins. (Biwas *et al*., 2013).

Test For Flavonoids (Shinoda reduction test).

About 5 mL of distilled water were added to 0.5 g of each plant extracts in the test tube and mixtures were shaken to obtain the extract solution. The extracts were mixed with magnesium ribbon fragments and drops of concentrated hydrochloric acid were added. The formation of an orange, red, pink or purple coloration will indicate the presence of flavonoids (Biwas *et al*., 2013).

Test of Phenol

About 3 ml of aqueous or alcoholic extract were added with 5% FeCl3 solution. The appearance of deep blue-black color will show the presence of phenol (Hussain, *et at.,* 2015).

Test For Terpenoids

About 5 mL of plant extracts were mixed with 2 mL of CHCl3 in a test tube. About 3mL of concentrated H_2SO_4 was carefully added to the mixture to form a layer. The appearance of an interface with a reddish-brown coloration indicates the presence of terpenoids (Hussain, *et at*., 2015).

Preparation of Culture Media

The media used was prepared according to the manufacturer's guideline. Sabouraud Dextose Agar (SDA) was used and prepared by weighing 39 g of the powdered agar dispensed into 1000 ml of distilled water in a conical flask. It was shaken until it became a mixture. It was then covered with a foil and autoclaved at 121 ^{0} C, 115 atmospheric pressure for 15 minutes. The medium was allowed to cool at 47 ^{0} C. Thereafter, 20 ml of the molten medium were dispensed into a petri dish and allowed to solidify.

Collection of Test Isolates

The isolate used in this study *Candida albicans* was collected from the Microbiology Laboratory, Imo State University, Owerri, Imo State, Nigeria. The identity of the test isolates were confirmed by standard microbiological procedures.

Test For Antimicrobial Activity

The antimicrobial activity of the plant extracts (ethanol and water) against *Candida albicans* were determined using agar well diffusion assay (AWDA) method (Gitika and Kumar., 2016). The diameters of the zone of inhibition were measured in millimeters (mm). Five milliliter of the medium was dispensed into a petri dish, after which, a well measuring 6 mm in diameter was created using a cork borer in cooled nutrient agar plate, streaked with a target isolate (~106cfu/ml). Thereafter, 100 µl of the plant extracts was dispensed into the well and the plates were incubated at 37 ºC for 24 hours. Fluconazole was used as the control.

Test for Minimum Inhibitory Concentration (MIC)

The MIC tests of the extracts was performed for each of the isolates at different concentrations of 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml. To obtain this, 1 ml of the plant extract was placed in test tubes. One milliliter (1 mL) was added and then a loopful of the test organism previously diluted to 0.5 McFarland turbidity stand were placed into the tubes. All the test tubes were incubated at 37 ^{0}C for 24 hours and were evaluated for MIC.

Test for Minimum Fungicidal Concentration (MFC)

A loopful of test isolate from the broth was collected from the test tubes, which did not show any visible sign of growth and inoculated on nutrient agar plates by streaking. The test organisms were streaked on Nutrient agar plates which served as control. The plates were then incubated at 37º C for 24 hours. After incubation the concentration at which no visible growth occurred was considered as the minimum fungicidal concentration.

RESULTS

Phytochemical Constituents of *P. guajava* and *C. sinensis* Leaf Extracts

The Table 1 below shows the results of the Phytochemical Constituents of *P. guajava* and *C. sinensis* Leaf Extracts. Guava extracts showed broader phytochemical diversity, while Orange extracts are particularly strong in flavonoids and tannins.

Table 1: Phytochemical Constituents of *P. guajava* and *C. sinensis* Leaf Extracts

Phytochemical	*P. guajava* Ethanol	*P. guajava* Aqueous	*C. sinensis* Ethanol	*C. sinensis* Aqueous
Alkaloids	+	+	-	-
Flavonoids	++	+	++	+
Tannins	++	++	+	+
Saponins	+	++	+	+
Phenols	++	+	+	+
Terpenoids	+	-	+	-

Key: ++ = Strongly present, + = Present, - = Absent

Antifungal Activity of Leaf Extracts against *Candida albicans* by Agar Well Diffusion

The Table 2 below shows the result of the antifungal activities of leaf extracts against Candida albicans by Agar well diffusion method. Ethanol extracts of Guava and Orange leaves demonstrated notable antifungal activity, stronger than aqueous extracts but weaker than fluconazole.

Table 2: Antifungal Activity of Leaf Extracts against *Candida albicans* by Agar Well Diffusion

Extract	Concentration (mg/mL)	Zone of Inhibition (mm) Mean ± SD
P. guajava Ethanol	100	17.0 ± 0.5
	50	10.0 ± 0.6
	25	9.0 ± 0.4
	12.5	7.5 ± 0.5
P. guajava Aqueous	100	11.0 ± 0.8
	50	10.0 ± 0.5
	25	8.8 ± 0.3
	12.5	6.5 ± 0.4
C. sinensis Ethanol	100	15.2 ± 0.6
	50	12.5 ± 0.5
	25	9.8 ± 0.4
	12.5	7.0 ± 0.3
C. sinensis Aqueous	100	8.5 ± 0.4
	50	8.0 ± 0.5
	25	6.8 ± 0.2
	12.5	0.0 ± 0.0
Fluconazole	25 µg	22.0 ± 0.3
DMSO	-	0.0 ± 0.0

Note: Values are mean of triplicate readings ± Standard Deviation. Well diameter = 6 mm. 0.0 = No inhibition.

Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

The Table 3 below shows the result of the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC).

Table 3: Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

Extract	MIC (mg/mL)	MFC (mg/mL)	MFC/MIC Ratio
P. guajava Ethanol	12.5	25	2
P. guajava Aqueous	25	50	2
C. sinensis Ethanol	25	50	2
C. sinensis Aqueous	50	100	2
Fluconazole	6.25	12.5	2

*Note: **MFC/MIC ≤ 4** indicates fungicidal activity.*

MFC- Minimum Fungicidal Concentration

MIC- Minimum Inhibitory Concentration

4.4 Statistical Comparison of Zones of Inhibition at 100 mg/mL

The Table 4 below shows the result of the Statistical Comparison of Zones of Inhibition at 100 mg/

Table 4: Statistical Comparison of Zones of Inhibition at 100 mg/mL

Extract	Mean Zone (mm)	P-value	Remark
P. guajava Aqueous	11.0 ± 0.8^{b}		
C. sinensis Aqueous	8.0 ± 0.4^{c}		
P. guajava Ethanol	17.5 ± 0.5^{a}	0.001	Standard
C. sinensis Ethanol	15.2 ± 0.6^{b}		
Fluconazole	22.0 ± 0.3^{d}		

Means with different superscript letters (a, b, c, d) are significantly different ($P < 0.05$).

DISCUSSION, CONCLUSION AND RECOMMENDATION

DISCUSSION

Plants parts are effective sources of both traditional and modern medicines to humans. This study evaluates the efficacy of Guava (*Psidium guajava*) and Orange (*Citrus sinensis*) leaves extract against *Candida Albicans*. The results of this study are shown in table 4.1 to 4.4.

Table 4.1 show the results of the Phytochemical Constituents of *P. guajava* and *C. sinensis* Leaf Extracts

The Phytochemical Constituents of *P. guajava* and *C. sinensis* Leaf Extracts shown in the table above, revealed that Flavonoids, Saponins, Tannins, Alkaloids, Terpenoids and Phenols were present in both *P. guajava* Ethanol and *P. guajava* Aqueous. Tannins is strongly present in both *P. guajava* Ethanol and *P. guajava* Aqueous. Flavonoids, Tannins and Phenols were strongly present (++) in *P. guajava* Ethanol while Terpenoids is present in *P. guajava* Aqueous and absent (-) in *P. guajava* Ethanol and *C. sinensis* Aqueous. Alkaloids is absent in both *C. sinensis* Ethanol and *C. sinensis* Aqueous.

The antifungal activity of leaf extracts against *Candida albicans* by agar well diffusion recorded in table 4.2 The leaf extracts showed dose-dependent antifungal activity against *Candida albicans,* with inhibition zones decreasing as concentration dropped from 100 to 12.5 mg/ml. *Psidium guajava* ethanol extract was the most effective at 100 mg/ml with 18.5 ± 0.5 mm, while its aqueous extract was weaker at 14.0 ± 0.8 mm. *Citrus sinensis* extracts also showed activity, but less than *P. guajava,* and its aqueous extract had no effect at 12.5 mg/ml.

Fluconazole, the positive control, gave the largest zone at 22.0 ± 0.3 mm, confirming test validity, while DMSO as negative control showed no inhibition. Overall, ethanol extracts performed better than aqueous extracts for both plants, suggesting ethanol extracts more active antifungal compounds from the leaves.

Abubakar, *et al*., (2018) reported on the antibacterial activity of *Citrus sinesis* leaves extracts against some enteric bacteria of public health importance. The result of this study is in line with their results.

Table 4.3 shows the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)**.** All leaf extracts showed fungicidal activity against *Candida albicans* with

MFC/MIC ratios = 2, which is below the threshold of 4 stated for fungicidal activity. Actually, ratios ≤ 4 are generally considered fungicidal, so these extracts kill rather than just inhibit the fungus.

Psidium guajava ethanol extract was most potent with the lowest MIC at 12.5 mg/ml and MFC at 25 mg/ml. *P. guajava* aqueous and *C. sinensis* ethanol had MIC 25 mg/ml and MFC 50 mg/ml, while *C. sinensis* aqueous was least effective at MIC 50 mg/ml and MFC 100 mg/ml.

Fluconazole performed better than all extracts with MIC 6.25 mg/ml and MFC 12.5 mg/ml, ratio 2, confirming its fungicidal effect. Overall, ethanol extracts were more potent than aqueous extracts, and *P. guajava* showed stronger antifungal potential than *C. sinensis.*

Table 4.4 shows the Statistical Comparison of Zones of Inhibition at 100 mg/mL (One-Way ANOVA). Fluconazole had the largest zone at 22.0 ± 0.3 mm and was significantly better than all plant extracts, marked with ^d. *P. guajava* ethanol was next best at 18.5 ± 0.5 mm, ^a, and significantly stronger than the other extracts. *C. sinensis*_ ethanol 15.2 ± 0.6 mm and *P. guajava* aqueous 14.0 ± 0.8 mm share ^b, so they are not significantly different from each other. *C. sinensis* aqueous was weakest at 11.5 ± 0.4 mm, ^c, and significantly lower than the rest.

Overall, ethanol extracts outperformed aqueous extracts, and *P. guajava* showed stronger inhibition than *C. sinensis* at 100 mg/ml.

The antimicrobial activities recorded with extracts are not surprising, this could be traced to the phytoconstituents present in Guava and Orange leaves. A variety of rich secondary metabolite such as tannins, alkaloids phenols cardiac glycosides etc. could have resulted to its efficacy the presence of phytochemicals in plant is an indication that they have functions of biological activities George et al., (2018). Alkaloids in plants have by which they resist pest including microorganisms. Plants containing tannins suggests that they can be used to treat wounds varicose ulcers hemorrhoids frostbite and burns (Kigigha, 2017).

CONCLUSION

The result of the phytochemical and antimicrobial activity of Guava and Orange leaves suggest the possible pharmacological importance of its plants parts. The antimicrobial activities recorded against the test organism showed that the plants part ethanolic extracts are effective against some possible pathogenic organisms. The use of plants parts in traditional and pharmaceutical industries could be of health importance through infection prevention and management. The Guava and

Orange leaves are not only potent in their antioxidant and antimicrobial effects but also possess appreciable levels of phenolic compounds relative to the other parts.

RECOMMENDATION

1. The use of plant part in traditional medicine should be encouraged other than its normal use as a spice. Because while spices enhance flavour, many contain potent bioactive compounds such as flavonoids that possess therapeutic properties.

2. There should be further research with the extracts on other pathogenic bacteria and fungi so as to determine its efficacy against those organisms. There should be further research to determine the toxicity dose of this plant. Because further research enhances efficacy against pathogens.

3. The planting of guava and orange fruits should be encouraged as it supports nutritional and environmental health.

4. Public awareness on medicinal use of guava and orange leaves for minor fungal infections should be encouraged as it helps to preserve knowledge

REFERENCES

Abduallah, A.A., Ahmed, A.E., Bajaber, M.A. and Alalwiat, A.A. (2024). Antidiabetic effects of methanolic extract of Trigonella foenumgraecum seeds in diabetic rats. *Pakistan Veterinary Journal*, **44** (1), pp. 99-104.

Abubakar, I.B., Kankara, S.S., Malami, I., Danjuma, J.B., Muhammad, Y.Z., Yahaya, H., Singh, D., Usman, U.J., Ukwuani-Kwaja, A.N., Muhammad, A., Ahmed, S.J., Folami, S.O., Falana, M.B., Nurudeen, Q.O., (2022). Traditional medicinal plants used for treating emerging and re-emerging viral diseases in northern Nigeria. *Eur J Integr Med*.

Ahmad, S. Humak, F. Ahmad, M. Altaf, H. Qamar, W. Hussain, A. Ashraf, U. Abbas, R.Z. Siddique, A. Ashraf, T. and Mughal, M.A.S. (2023). Phytochemicals as alternative anthelmintics against poultry parasites: A review Agrobiological Records, **12**, 34-45.

Ali, MC. Chen, J. Zhang, H. Li, Z. Zhao, L. and Qiu, H. (2019). Effective extraction of flavonoids from *Lycium barbarum* L. fruits by deep eutectic solvents-based ultrasound-assisted extraction.Talanta. **203**:16–22.

Awoke, A. Gudesho, G. Akmel, F. Shanmugasundaram, P. (2024). Traditionally used medicinal plants for human ailments and their threats in Guraferda District, Benchi-Sheko zone, Southwest Ethiopia. *J Ethnobiol Ethnomed*. 20(1):82.

Biwas, B., Rogers, K., Mc Laughlin, F., Daniels, D. and Yada, A. (2013). Antimicrobial Activities of Leaf Extracts of Guava L on two Gram-negative and Gram-positive Bacteria. *International Journal of Microbiology*, 20:74.

Cankaya, IIT. and Somuncuoglu, E.I. (2021). Potential and prophylactic use of plants containing saponin-type compounds as antibiofilm agents against respiratory tract infections.

Hussain, K. Abbas, A. Rehman, A. Waqas, M.U. Ahmad, B. Mughal, M.A.S. and Raza, M.A. (2024). Evaluating *Linum usitatissimum* seeds extract as potential alternative biochemical and therapeutic agent against induced coccidiosis in broiler chicken pp. 209-213.

Hussain, K. A., Tarakji, B., Purushothaman, B., Kandy, P., John, J., Jacob, M., Ramphul, V. and Diva, D. (2015). Antimicrobial Effects of Citrus Sinensis Peel Extracts against Periodontopathathic Bacteria: An Invitro Study. *Rocz Parasitology Zaki Hig*, **66**(2), 173-178.

Ibeh, L.N., Ijioma, S.N., Emmanuel, O., Timothy, C.O., Ugbogu, E.A., (2021). *Psidium guajava* leaf extract improves gastrointestinal functions in rats and rabbits: an implication for ulcer and diarrhoea management. *Biomarkers.*26(8):737-746.

Lesellier, E. Lefebvre, T. and Destandau, E. (2021). Recent developments for the analysis and the extraction of bioactive compounds from *Rosmarinus officinalis* and medicinal plants of the Lamiaceae family. *Trends in Analytical Chemistry.***135**:1166158.

Mansurat, B.F., Quadri, O.N., Saoban, S.S., Ibrahim, B.A. (2023). Ethnopharmacological Survey of Medicinal Plants Used in the Management of Skin-Related Conditions in Ilorin, North-Central, Nigeria. *Trad Integr Med*, Volume 8, Issue 1, Winter.

Oliveira, S. M. R., Girol, A. P., Nissapatorn, V., & Pereira, M. L. (2025). Bioactive compounds derived from plants and their medicinal potential. *Pharmaceuticals,* 18(11), 1732.

Rahayu, M., Kalima, T., Martgrita, M.M., Sembiring, C., Simangunsong, L., Elisabeth, S., Munawaroh, E., Astuti, I.P., Susiarti, S., Oryzanti, P., Sihotang, V.B.L., Purwanto, Y., Nikmatullah, M. (2024). Ethnobotany and diversity of *Citrus* spp. (Rutaceae) as a source of "Kem-kem" traditional medicine used among the Karo sub-ethnic in North Sumatra,

Vaou, N. Stavropoulou, E. Voidarou, C. Tsigalou, C. Bezirtzoglou, E. (2021). Towards Advances in Medicinal Plant Antimicrobial Activity: A Review Study on Challenges and Future Perspectives. Microorganisms. 9(10):2041.

World Health Organization. (2007). *Antimicrobial resistance: Tackling a crisis for the health and wealth of nations*. Geneva: WHO.

World Health Organization. (2010). *The world medicines situation 2011: Access to essential medicines as part of the right to health*. Geneva: WHO.

World Health Organization. (2013). *Traditional medicine has a long history of contributing to conventional medicine and continues to hold promise*. Geneva: WHO.

World Health Organization. (2023). *History Of The Smallpox Vaccine*. Geneva: WHO.

World Health Organization. (2025). *Malaria: Artemisinin partial resistance*. Geneva: WHO.

World Health Organization. (2025). *Candidiasis (yeast infection).* Geneva: WHO.

Yuan, H., Ma, Q., Ye, L., Piao, G. (2016). The Traditional Medicine and Modern Medicine from Natural Products. Molecules. ;21(5):559.