Antibacterial Potency of Fractions from *Psidium guajava* Leaves and Bark Crude Extracts against Bacterial Isolates Responsible for Foodborne Infection

JAMES A. NDAKO * , , Emmanuel O Oludipe , Surajudeen A. Junaid , Rachael F. Echemita , Victor O. Fajobi , Victor T. Dojumo , Precious J. Ndako , Adedamola O. Omole

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Article

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James A. Ndako [1,2,3,*], Emmanuel O. Oludipe [1,2,3], Surajudeen Alim Junaid [1,4], Rachael F. Echemita [1,2,3], Victor O. Fajobi [1,5], Victor T. Dojumo [1,5] Precious J. Ndako [1,2,3] and Adedamola O. Omole [1,2,3]

[1] Landmark University SDG 3 (Good Health and Well-Being Research Group); manueloludipe@gmail.com (E.O.O.); .junaid@medicine.fulafia.edu.ng (S.A.J.); echemita.rachael@lmu.edu.ng (R.F.E.); fajobi.victor@lmu.edu.ng (V.O.F.); dojumo.victor@lmu.edu.ng (V.T.D.); preshystar15@gmail.com (P.J.N.); damolaomole123@gmail.com (A.O.O.)
[2] Landmark University SDG 11 (Sustainable Cities and Communities Research Group)
[3] Department of Microbiology, Landmark University Omu-aran, Nigeria.
[4] Department of Medical Laboratory Sciences, Federal University, Lafia.
[5] Department of Medical Laboratory Services, Landmark University Medical Center, Omu-aran
* Correspondence: Author: ndako.james@lmu.edu.ng; 07038540403

ABSTRACT: Guava (*Psidium guajava* L.) is a common fruit tree that grows in several tropical and subtropical parts of the world. The aim of this study was to employ the use of liquid-liquid fractionation to investigative the comparative antibacterial potential of crude extracts of Guava leaves and bark against selected food isolates; *Escherichia coli, Pseudomonas aeruginosa, Streptococcus pneumonia, Bacillus cereus* and *Staphylococcus aureus*. The phytochemical analysis of the extract showed presence of tannin, phenol, flavonoid and terpenoid in all extract, while steroid and saponin were absent in some. The agar diffusion method was employed for the assessment of the sensitivity of the extracts. The ethyl acetate and aqueous fractions from the stem bark acetone extract generally showed better antimicrobial activity compared with other extracts from leaves. The extract was active both against gram positive bacteria (*Bacillus cereus and Streptococcus pneumonia*) and gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, , Bacillus cereus* and *Staphylococcus aureus*) at varying zone of inhibition. The results of the study showed the potential of identifying novel antibacterial agent from *P. guajava* bark and leaves while optimising the potential application for treatment in traditional medicine.

Keywords: antibacterial activity, *Psidium guajava*; phytochemical screening; liquid-liquid fractionation

INTRODUCTION

Foodborne disease resulting from bacterial contamination during food processing or consumption of spoilt food are a common cause of illness and death especially in developing countries [1,2]. Common foodborne pathogenic bacteria such as *Escherichia coli, Bacillus cereus, Listeria monocytogenes Salmonella* Typhi and *Staphylococcus aureus* among others have been well documented to produce toxins which result in diverse range of disease condition [3]. Incidences of community food borne disease outbreaks are frequently reported in low and middle income countries, with children been most vulnerable [4]. Food preservation plays critical role in the control of food borne diseases. However, the conventional use of chemicals as preservatives have been faulted as possessing harmful effect on human health [5–7]. Likewise, treatment of infectious disease conditions with commercial antibiotic has proven less effective due to the increasing incidence of antimicrobial resistance [8]. Conventional medication are also relatively expensive for treatment in low and

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medium income economies [9]. These combined concerns has prompted the intense research and development of alternatives for food preservation and treatment of food borne disease. Several emerging methods have being developed. Of these, the traditional plant based approach to food borne disease treatment, food preservation and packaging have received great attention for safety, antimicrobial efficiency and biodegradability [2,10,11]. In addition to it inexpensive cost and accessibility, it therefore remains plausible to consider ethnomedicinal plants as potential source of antimicrobial for food preservation and treatment of food borne disease.

Guava (*Psidium guajava* L.) is a common fruit tree that grows in several tropical and subtropical parts of the world. It is widely valued because of its edible fruit and its leaves [12,13]. The Guava leaves are used traditionally for treatment of several ailments around the world. In West Africa, the leaves are utilised as a key recipe in herbal preparation for the treatment of malaria which is endemic to the region. It is reported to contain phytochemicals such as flavonoids, carotenoids and polyphenols among others [14]. Quercetin has been isolated as one of the major compounds in the leaves [15]. The ethnobotanical use and bioactivity of Guava leaves includes but not limited to wound healing, antidiabetic, as cough sedative, anti-inflammatory etc. [16]. The twig of the Guava plant is popularly used as chewing stick in Western Nigeria which is used in cleaning by brushing against the teeth and gum [17]. Use of this local chewing stick and extracts has been reported to reduce present of tartar/dental plaque formation in the mouth and investigated for it antibacterial activities, especially as directed to oral health [18]. Because of the potentials of the different Guava parts, the leaves and stem bark have been the focus of several antimicrobial study. It close antibacterial activity against food related pathogen occurring in the mouth makes it a potential plant based alternative for food preservation and treatment of disease condition resulting from food poisoning. However, there is yet to be an exhaustive study for comparing the bioactivity of the plant extract based on polarity. As any part of a plant can contain natural bioactive constituents, likewise the polarity of extracts could significantly influence it activity. Constitute of some plant phytochemical can complex and buffer the activity of others. Therefore the aim of this study was to employed the use of liquid-liquid fractionation to investigative the comparative antibacterial activity of crude extracts of Guava leaves and bark against selected food isolates; *Escherichia coli, Pseudomonas aeruginosa, Streptococcus pneumonia*, *Bacillus cereus* and *Staphylococcus aureus*.

MATERIALS AND METHODS

Plant Material

Psidium guajava leaves and stem bark were harvested from Guava trees at the Orchard Garden, Landmark University, Omu-aran, Kwara State (8°07'30.9"N; 5°04'53.8"E). The plant samples were rinsed under running tap water. Part of the collected fresh leaves were stored at 4°C and the remaining plant sample air dried. The dried materials were pulverised with an electric blender and stored in a labelled air-tight container until use.

Crude Extract Preparation

Approximately 300 g of pulverized dried leaves were extracted with 1500 mL of distilled water, acetone and ethanol in Erlenmeyer flasks, same was done for the fresh leaves. Similarly, 200g of dried stem bark was macerated in 1000 mL of distilled water and acetone. The setup was wrapped in aluminium foil to avoid evaporation and exposure to light and then placed on a Rocking Laboratory Shaker for 72 hours agitation at 70 rpm. Subsequently the mixture was decanted and filtered through a Whatsman filter paper [19]. The filtrate was concentrated using a Rotary Evaporator, dried at 45ºC and stored in the refrigerator at 4°C until use.

Fractionation of Crude Extracts

For each crude extract, 20 g was reconstituted in 100 mL distilled water and agitated for 10 minutes. The mixture was transferred to s separating funnel, where 300 mL n-Hexane was added, shaken vigorously and allowed to partition for 24 hours. The n-Hexane fraction was collected in sterile container and the procedure for partition was repeated to collect ethyl acetate fraction. The solvent from fractions were evaporated at 45°C in a water bath [20].

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Qualitative Phytochemical Screening

Test for phytochemicals in crude *Psidium guajava* leaves and stem bark extract was analysed for saponins, tannins, phenols, flavonoids, terpenoids and steroids using standard protocols [21,22]

Organisms used in study

The test bacteria isolates used in this experimental study were isolated from food sample. They were identified by morphological, cultural, and biochemical characteristics. The list of isolates include; two strains of Gram negative (*Escherichia coli*, and *Pseudomonas aeruginosa*) and three strains of Gram positive (*Staphylococcus aureus*, *Streptococcus pneumonia* and *Bacillus cereus*) bacteria. Fresh culture of each isolates was utilized in assessing antibacterial activity of the Guava leaf and stem bark crude extracts.

Antibacterial activity of extracts

A stock preparation of 100 mg/mL of each plant extract fraction was prepared in solution of 10% DMSO. The agar well diffusion technique was used in assessing antibacterial properties of each fraction. For each test organism, a fresh 18 to 24 hour culture which was plated on Nutrient Agar was utilised as inoculum at 0.5MarFarland (containing about 1.5×10^8 CFU/mL) in normal sterile saline water [23]. The inoculum was swapped on a freshly prepared solidified Muller Hinton Agar prepared in accordance with the manufacturer's instruction. Wells of 9 mm were made in the inoculated Muller Hinton Agar using a cork borer. The wells were filled with the prepared test fraction and ciprofloxacin as a positive control. The cultures were incubated at 37 ºC for 24 hours. The diameter of zone of inhibition around the wells was recorded as level of antibacterial activity. Each assay was carried out in replicate.

Statistical analysis

Result of zones of inhibition was analysed using IBM-SPSS Statistics version 26 (IBM Corp., USA) software and reported as mean ± standard deviation ($p < 0.05$).

RESULTS

The results of qualitative phytochemical screening analysis of Guava leaf and stem bark extracts are presented in Table 1. All the extracts were present for Tannin, Phenol, Flavonoid and Terpenoids. Saponin was present in the aqueous leaf extract and the stem bark aqueous and acetone extract, but was absent in the leaf ethanol and acetone extracts. The stem bark showed absence of Steroids. However, steroids was present in the leaf extracts excluding acetone leaf extract.

Table 1. Qualitative analysis of Guava (*Psidium guajava*) leaf and stem bark extracts.

Phytochemicals	Leaf extract			Stem bark extract	
	Aq	*EtOH*	*Ace*	*Aq*	*Ace*
Saponin	+	-	-	+	+
Tannin	+	+	+	+	+
Phenol	+	+	+	+	+
Flavonoid	+	+	+	+	+
Terpenoid	+	+	+	+	+
Steroids	+	+	-	-	-

Key: (+) = Present; (−) = Absent

The antibacterial activity of the fraction of extracts from *Psidium guajava* leaves and stem bark against *E. coli* (29.0 ± 1.0 mm to 14.0 ± 1.0 mm) is shown in Table 2 compared to the standard antibiotics Ciprofloxacin (53.0 ± 1.0mm). Based on zone of inhibition, the widest inhibition diameter was reported for Ethyl Acetate fraction of Stem bark acetone extract at 29.0 ± 1.0 mm, followed by 23.5 ± 1.5 mm in n-Hexane fraction of Fresh leaf aqueous extract and 21.0 ± 1.0 mm in Ethyl Acetate faction

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of Dried Leave ethanol extract. The least activity was reported for Ethyl Acetate fraction Fresh leaf aqueous extract (14.0 ± 1.0 mm).

Table 2. Antibacterial activity of Fractions from Guava (*Psidium guajava*) leaves and stem bark crude extracts and against *Escherichia coli.*

Crude Extract		Fractions (100 mg/mL)		
		n-Hexane	Ethyl Acetate	Water
Dried Leaves	*Aq*	16.5 ± 1.5	18.0 ± 1.0	19.5 ± 0.5
	EtOH	20.0 ± 2.0	21.0 ± 1.0	15.5 ± 0.5
	Ace	20.5 ± 1.5	19.5 ± 0.5	17.0 ± 1.0
Fresh Leaves	*Aq*	23.5 ± 1.5	14.0 ± 1.0	16.5 ± 1.5
Stem Bark	*Aq*	-	-	17.0 ± 1.0
	Ace	16.5 ± 1.5	29.0 ± 1.0	17.0 ± 1.0
Ciprofloxacin (Control)		53.0 ± 1.0	53.0 ± 1.0	53.0 ± 1.0

Test of fractionated crude extract from *Psidium guajava* leaves and stem bark against *P. aeruginosa* is shown in Table 3, with activity ranging from 34.0 ± 1.0 mm to 13.5 ± 1.5 mm. n-Hexane fraction of dried leaf acetone extract had the largest zone of inhibition (34.0 ± 1.0 mm). Stem bark acetone extract had activity at 27.5 ± 2.5 mm for it aqueous fraction and 26.1 ± 1.00 mm for ethyl acetate fraction. The water fraction of the dried leave aqueous extract was least in zone of inhibition at 13.5 ± 1.5 mm.

Table 3. Antibacterial activity of Fractions from Guava (*Psidium guajava*) leaves and stem bark crude extracts and against *Pseudomonas aeruginosa.*

Crude Extract		Fractions (100 mg/mL)		
		n-Hexane	Ethyl Acetate	Water
Dried Leaves	*Aq*	20.0 ± 0.0	15.5 ± 1.5	13.5 ± 1.5
	EtOH	19.0 ± 1.0	24.0 ± 1.0	17.0 ± 0.0
	Ace	34.0 ± 1.0	24.0 ± 1.0	16.5 ± 1.5
Fresh Leaves	*Aq*	23.5 ± 1.5	11.5 ± 0.5	14.0 ± 1.0
Stem Bark	*Aq*	-	-	19.0 ± 1.0
	Ace	15.0 ± 1.0	26.0 ± 1.0	27.5 ± 2.5
Ciprofloxacin (Control)		51.0 ± 1.5	51.0 ± 1.5	51.0 ± 1.5

Result of inhibitory activity of fractions from Guava (*Psidium guajava*) leaves and stem bark crude extracts and against *Streptococcus pneumonia* ranged from 27.5 ± 1.5 mm to 11.5 ± 0.5 mm, where the control Ciproflaxin was 41.5 ± 1.5 mm. Ethyl Acetate fraction of Dried leaf ethanol extract ant Stem bark acetone extract showed antibacterial activity against *Streptococcus pneumonia* at 27.5 ± 1.5 mm and 27.0 ± 1.0 mm respectively (Table 4). The ethyl acetate and aqueous fractions for dried leaf acetone extract both exhibited activity at 21.0 ± 1.0 mm against *Streptococcus pneumonia*. The least activity (11.5 ± 0.5 mm) was recorded for n-Hexane fraction of Fresh leaf aqueous extract.

Table 4. Antibacterial activity of Fractions from Guava (*Psidium guajava*) leaves and stem bark crude extracts and against *Streptococcus pneumonia.*

Crude Extract		Fractions (100 mg/mL)		
		n-Hexane	Ethyl Acetate	Water
Dried Leaves	*Aq*	12.0 ± 1.0	16.5 ± 1.5	21.0 ± 1.0
	EtOH	13.5 ± 0.5	27.5 ± 1.5	25.5 ± 3.5
	Ace	16.5 ± 0.5	26.0 ± 1.0	26.0 ± 1.0
Fresh Leaves	*Aq*	11.5 ± 0.5	21.0 ± 1.0	24.0 ± 0.0
Stem Bark	*Aq*	-	-	22.5 ± 0.5

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	Ace	19.5 ± 0.5	27.0 ± 1.0	24.5 ± 0.5
Ciprofloxacin (Control)		41.5 ± 1.5	41.5 ± 1.5	41.5 ± 1.5

The antibacterial activity of the fraction of crude extract from *Psidium guajava* against *Bacillus cereus* ranged from 29.0 ± 1.0 mm to 12.0 ± 1.0 mm, with the control antibiotic (Ciprofloxain) 59.0 ± 1.0 mm (Table 5). Ethyl acetate fraction from Stem bark acetone extract showed the highest inhibition against *Bacillus cereus* at 29.0 ± 1.0 mm. Stem bark aqueous extract was 24.5 ± 0.5 mm and aqueous fraction of dried leaf ethanol extract at 24.0 ± 1.0 mm. The least activity was in aqueous fraction of fresh leave aqueous extract (12.0 ± 1.0 mm).

Table 5. Antibacterial activity of Fractions from Guava (*Psidium guajava*) leaves and stem bark crude extracts and against *Bacillus cereus.*

Crude Extract		**Fractions (100 mg/mL)**		
		n-Hexane	Ethyl Acetate	Water
Dried Leaves	*Aq*	13.0 ± 1.0	23.0 ± 1.0	17.0 ± 1.0
	EtOH	23.0 ± 1.5	20.5 ± 0.5	24.0 ± 1.0
	Ace	16.0 ± 1.0	19.5 ± 0.5	23.0 ± 1.0
Fresh Leaves	*Aq*	19.5 ± 0.5	20.0 ± 2.0	12.0 ± 1.0
Stem Bark	*Aq*	-	-	24.5 ± 0.5
	Ace	16.5 ± 1.5	29.0 ± 1.0	23.5 ± 1.5
Ciprofloxacin (Control)		59.0 ± 1.0	59.0 ± 1.0	59.0 ± 1.0

The range of inhibition of fractions from *Psidium guajava* leaves and stem bark crude extracts was from 19.5 ± 0.5 mm to 30.0 ± 1.0 mm against *Staphylococcus aureus*. The control antibiotic was 43.5 ± 0.5 mm in zone of inhibition (Table 6). The widest zone of inhibition was observed in aqueous fraction of Stem bark acetone extract (30.0 ± 1.0 mm). Aqueous fractions of dried leaf Ethanol and acetone extract show inhibition at 29.5 ± 0.5 mm and 26.5 ± 0.5 mm respectively. The least activity was reported for aqueous fraction of dried leaf aqueous extract at 19.5 ± 0.5 mm.

Table 6. Antibacterial activity of Fractions from Guava (*Psidium guajava*) leaves and stem bark crude extracts and against *Staphylococcus aureus.*

Crude Extract		**Fractions (100 mg/mL)**		
		n-Hexane	Ethyl Acetate	Water
Dried Leaves	*Aq*	22.0 ± 1.0	21.5 ± 1.5	19.5 ± 0.5
	EtOH	20.5 ± 0.5	25.0 ± 1.0	29.5 ± 0.5
	Ace	22.0 ± 1.0	23.0 ± 0.0	26.5 ± 0.5
Fresh Leaves	*Aq*	24.5 ± 0.5	21.0 ± 2.0	23.0 ± 2.0
Stem Bark	*Aq*	-	-	20.5 ± 0.5
	Ace	22.0 ± 2.0	26.0 ± 1.0	30.0 ± 1.0
Ciprofloxacin (Control)		43.5 ± 0.5	43.5 ± 0.5	43.5 ± 0.5

DISCUSSION

According to the WHO Traditional Medicine Strategy (2014-2023), traditional medicine has continued to play a vital role in treatment and management of diseases, especially in primary health care [24,25]. Therefore medicinal plants are continuously being sourced from the environment and prepared into herbal products. Different techniques are employed in this preparation of which include but are not limited to maceration, infusion, decoction, hot steam extraction among others [26]. For efficiency in plant extraction and yield, increasing the surface area of solvent and sample is important. The solvent type also has a complementary effect on the combination of active ingredients to be extracted [27].

This study reports on the presence of phytochemicals containing the active component of the plant extract. The choice of extraction solvent was based on availability, sustainable utilisation and report of prior antibacterial activity from other studies [28–30]. Water which is polar and is commonly used in traditional settings for extraction of plant bioactive components in the treatment of disease conditions. Ethanol and Acetone are solvent of mid polarity. While ethanol is cheap and likewise commonly use similarly as water in folk medicine, studies have reported acetone to be capable of isolating antimicrobial compounds with high activity. Acetone as a solvent also has an advantage of easy removal from solution compared to water and ethanol [31,32]. Likewise acetone has be reported to be nontoxic to bioassay systems and generally have high antimicrobial activities in several studies [33]. Results of this study qualitative phytochemical screening showed a wide range of compounds present in both *Psidium guajava* leaf and stem bark (Table 1). This included Tanin, Phenol, Flavonoid and Terpenoid, this is consistent to other reports [34]. However, absence of Saponin in Leaf ethanol and acetone extract shows effect of solvent polarity on type of phytocompound extracted. Likewise, both extract from the stem bark for aqueous and acetone was absent for Steroids. Liquid-liquid fraction is a technique for partitioning the range of compounds in a mixture based on polarity [35]. During the fractions of the crude extracts from *Psidium guajava* leaf and stem bark, choice of solvent was based on polarity. n-Hexane is a non polar solvent, regularly used for the defatting process of plant extracts. Ethyl acetate is a mid polar solvent and has the capacity to elute compounds in its mid polarity range. The liquid liquid fractionation process resulted in varying amount of fractions except in the stem bark aqueous extract, where the n-Hexane and ethyl acetate fraction did not result in any yield.

Food contamination may pose a great threat to the human population, especially in cases resulting from infectious diseases and food poisoning [36]. In this study the antibacterial potential of different fractions of *Psidium guajava* leaf and stem bark extracts was assessed against five (5) isolates from food sources. The isolates included *Escherichia coli, Pseudomonas aeruginosa, Streptococcus pneumonia*, *Bacillus cereus* and *Staphylococcus aureus*. The ethyl acetate and aqueous fractions from the stem bark acetone extract generally showed better antimicrobial activity compared with other extracts from leaves. For *Bacillus cereus and Streptococcus pneumonia* which are both gram positive bacteria and *Escherichia coli* (gram positive), the ethyl acetate fraction of the steam bark acetone extract showed the most activity. While the aqueous fraction of acetone extract was most inhibitory for *Staphylococcus aureus* (gram positive). This shows that phytocompounds in the stem bark has potential mechanisms of antimicrobial activity against both gram positive and negative bacteria. In the leaf extracts, n-Hexane fraction from dried leaf acetone extract showed highest activity for *Pseudomonas aeruginosa* (gram negative). In study acetone extracts generally have higher anti-microbial activity. Nevertheless, all fractions of extracts showed considerable bioactivity against the test bacteria isolates. The present study in addition have demonstrated the importance of extract fraction in optimising bioactivity.

CONCLUSION

This study shows the potential of identifying novel antibacterial agent from *Psidium guajava* leaf and stem bark, while optimising the potential application for treatment in traditional medicine. In light of latest trends in plant natural products research and development, green synthesis of nanoparticles from guava stem and leaf could aid increase antibacterial activity, for food, sustainable agriculture and treatment [37–39]. Following the WHA resolution on Traditional Medicine (WHA62.13) for safety toxicological analysis should be performed on the extract fractions in dose dependent manner. Further work is suggested to isolate antibacterial compounds and characterize them from the fractions especially the stem bark and analyse same for their therapeutic and optimal development into pharmaceutical products.

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Adedamola O. Omole- Responsible for Formal analysis

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REFERENCES

1. **Dawwam GE, Al-Shemy MT, El-Demerdash AS**. Green synthesis of cellulose nanocrystal/ZnO bio-nanocomposites exerting antibacterial activity and downregulating virulence toxigenic genes of food-poisoning bacteria. *Sci Rep* 2022;12:16848.
2. **Yassin MT, Abdel-Fattah Mostafa A, Al-Askar AA, Alkhelaif AS**. In vitro antimicrobial potency of Elettaria cardamomum ethanolic extract against multidrug resistant of food poisoning bacterial strains. *J King Saud Univ - Sci* 2022;34:102167.
3. **Takó M, Kerekes EB, Zambrano C, Kotogán A, Papp T, *et al.*** Plant Phenolics and Phenolic-Enriched Extracts as Antimicrobial Agents against Food-Contaminating Microorganisms. *Antioxidants* 2020;9:165.
4. **Cissé G**. Food-borne and water-borne diseases under climate change in low- and middle-income countries: Further efforts needed for reducing environmental health exposure risks. *Acta Trop* 2019;194:181–188.
5. **Abd El-Hack ME, El-Shall NA, El-Kasrawy NI, El-Saadony MT, Shafi ME, *et al.*** The use of black pepper (Piper guineense) as an ecofriendly antimicrobial agent to fight foodborne microorganisms. *Environ Sci Pollut Res* 2022;29:10894–10907.
6. **Petrova P, Arsov A, Tsvetanova F, Parvanova-Mancheva T, Vasileva E, *et al.*** The Complex Role of Lactic Acid Bacteria in Food Detoxification. *Nutrients* 2022;14:2038.
7. **Wu F, Rodricks JV**. Forty Years of Food Safety Risk Assessment: A History and Analysis. *Risk Anal* 2020;40:2218–2230.
8. **Laxminarayan R, Van Boeckel T, Frost I, Kariuki S, Khan EA, *et al.*** The Lancet Infectious Diseases Commission on antimicrobial resistance: 6 years later. *Lancet Infect Dis* 2020;20:e51–e60.
9. **Cameron A, Ewen M, Ross-Degnan D, Ball D, Laing R**. Medicine prices, availability, and affordability in 36 developing and middle-income countries: a secondary analysis. *The Lancet* 2009;373:240–249.
10. **Jafarzadeh S, Jafari SM, Salehabadi A, Nafchi AM, Uthaya Kumar US, *et al.*** Biodegradable green packaging with antimicrobial functions based on the bioactive compounds from tropical plants and their by-products. *Trends Food Sci Technol* 2020;100:262–277.
11. **Salanță LC, Cropotova J**. An Update on Effectiveness and Practicability of Plant Essential Oils in the Food Industry. *Plants* 2022;11:2488.
12. **Angulo-López JE, Flores-Gallegos AC, Torres-León C, Ramírez-Guzmán KN, Martínez GA, *et al.*** Guava (Psidium guajava L.) Fruit and Valorization of Industrialization By-Products. *Processes* 2021;9:1075.
13. **Rajan S, Hudedamani U**. Genetic Resources of Guava: Importance, Uses and Prospects. In: Rajasekharan PE, Rao VR (editors). *Conservation and Utilization of Horticultural Genetic Resources*. Singapore: Springer. pp. 363–383.
14. **Akkawi M, Abu-Lafi S, Abouremeleh Q, Lutgen P**. Screening of guava (Psidium guajava) leaves extracts against β-hematin formation. 9. Epub ahead of print 4 February 2021. DOI: 10.15406/ppij.2021.09.00319.
15. **Naseer S, Hussain S, Naeem N, Pervaiz M, Rahman M**. The phytochemistry and medicinal value of Psidium guajava (guava). *Clin Phytoscience* 2018;4:32.
16. **Kumar M, Tomar M, Amarowicz R, Saurabh V, Maheshwari C, *et al.*** Guava (Psidium guajava L.) Leaves: Nutritional Composition, Phytochemical Profile, and Health-Promoting Bioactivities. *Foods*. Epub ahead of print 1 April 2021. DOI: 10.3390/foods10040752.
17. **Kapoor S, Gandhi N, Kapoor A**. Guava (Psidium guajava). In: Nayik GA, Gull A (editors). *Antioxidants in Fruits: Properties and Health Benefits*. Singapore: Springer. pp. 227–249.
18. **Hassan SA, Metwalli NE, Ibrahim GG, Aly MA**. Comparison of the efficacy of mouth rinses camellia sinensis extract, guava leaves extract and sodium fluoride solution, on Streptococcus mutans and Lactobacillus in children (an in vivo study). *Future Dent J*. Epub ahead of print 27 November 2018. DOI: 10.1016/j.fdj.2018.11.002.
19. **Dada EO, Ekundayo FO, Makanjuola OO**. Antibacterial Activities of Jatropha curcas (LINN) on Coliforms Isolated from Surface Waters in Akure, Nigeria. *Int J Biomed Sci IJBS* 2014;10:25–30.
20. **Quintanilla-Licea R, Gomez-Flores R, Samaniego-Escamilla MÁ, Hernández-Martínez HC, Tamez-Guerra P, *et al.*** Cytotoxic Effect of Methanol Extracts and Partitions of Two Mexican Desert Plants against the Murine Lymphoma L5178Y-R. *Am J Plant Sci* 2016;7:1521–1530.
21. **Izuegbuna O, Otunola G, Bradley G**. Chemical composition, antioxidant, anti-inflammatory, and cytotoxic activities of Opuntia stricta cladodes. *PLOS ONE* 2019;14:e0209682.

22. **Owolabi A, Ndako J, Owa S, Oluyori A, Oludipe E, *et al.*** Antibacterial and Phytochemical Potentials of Ficus capensis Leaf Extracts Against Some Pathogenic Bacteria. Epub ahead of print 5 April 2022. DOI: 10.26538/tjnpr/v6i3.14.
23. **Noel D, Wain J, Lar P**. Use of Vitex doniana (black plum) and Abutilon hirtum (Florida keys) extracts as an integral part of phytomedicine in tackling multidrug-resistant Salmonella. *J Infect Dev Ctries* 2022;16:1323–1328.
24. **Liu C**. Overview on development of ASEAN traditional and herbal medicines. *Chin Herb Med* 2021;13:441–450.
25. **Savatagi SB, Srinivas PN, Payyappallimana U**. Factors influencing the emergence of self-reliance in primary health care using traditional medicine: A scoping review. *Indian J Public Health* 2022;66:214.
26. **Manousi N, Sarakatsianos I, Samanidou V**. 10 - Extraction Techniques of Phenolic Compounds and Other Bioactive Compounds From Medicinal and Aromatic Plants. In: Grumezescu AM, Holban AM (editors). *Engineering Tools in the Beverage Industry*. Woodhead Publishing. pp. 283–314.
27. **Chuo SC, Nasir HM, Mohd-Setapar SH, Mohamed SF, Ahmad A, *et al.*** A Glimpse into the Extraction Methods of Active Compounds from Plants. *Crit Rev Anal Chem* 2022;52:667–696.
28. **Mehmood A, Javid S, Khan MF, Ahmad KS, Mustafa A**. In vitro total phenolics, total flavonoids, antioxidant and antibacterial activities of selected medicinal plants using different solvent systems. *BMC Chem* 2022;16:64.
29. **Patra A, Abdullah S, Pradhan RC**. Review on the extraction of bioactive compounds and characterization of fruit industry by-products. *Bioresour Bioprocess* 2022;9:14.
30. **Rezaei M, Ghasemi Pirbalouti A**. Phytochemical, antioxidant and antibacterial properties of extracts from two spice herbs under different extraction solvents. *J Food Meas Charact* 2019;13:2470–2480.
31. **Eloff JN**. Avoiding pitfalls in determining antimicrobial activity of plant extracts and publishing the results. *BMC Complement Altern Med* 2019;19:106.
32. **Karaaslan MA, Cho M, Liu L-Y, Wang H, Renneckar S**. Refining the Properties of Softwood Kraft Lignin with Acetone: Effect of Solvent Fractionation on the Thermomechanical Behavior of Electrospun Fibers. *ACS Sustain Chem Eng* 2021;9:458–470.
33. **Famuyide IM, Aro AO, Fasina FO, Eloff JN, McGaw LJ**. Antibacterial and antibiofilm activity of acetone leaf extracts of nine under-investigated south African Eugenia and Syzygium (Myrtaceae) species and their selectivity indices. *BMC Complement Altern Med* 2019;19:141.
34. **Yahaya A, Ali M, Hassan FIE-, Jido BA**. Antibacterial activity of guava (Psidium guajava l.) extracts on Staphylococcus aureus isolated from patients with urinary tract infections attending a tertiary-care hospital. *Sci World J* 2019;14:47–51.
35. **Ho KL, Tan CG, Yong PH, Wang CW, Lim SH, *et al.*** Extraction of phytochemicals with health benefit from Peperomia pellucida (L.) Kunth through liquid-liquid partitioning. *J Appl Res Med Aromat Plants* 2022;30:100392.
36. **Garvey M**. Food pollution: a comprehensive review of chemical and biological sources of food contamination and impact on human health. *Nutrire* 2019;44:1.
37. **Jiang Y, Zhou P, Zhang P, Adeel M, Shakoor N, *et al.*** Green synthesis of metal-based nanoparticles for sustainable agriculture. *Environ Pollut* 2022;309:119755.
38. **Qamer S, Romli MH, Che-Hamzah F, Misni N, Joseph NMS, *et al.*** Systematic Review on Biosynthesis of Silver Nanoparticles and Antibacterial Activities: Application and Theoretical Perspectives. *Molecules* 2021;26:5057.
39. **Vieira IRS, de Carvalho APA de, Conte-Junior CA**. Recent advances in biobased and biodegradable polymer nanocomposites, nanoparticles, and natural antioxidants for antibacterial and antioxidant food packaging applications. *Compr Rev Food Sci Food Saf* 2022;21:3673–3716.