JOURNAL OF VETERINARY AND APPLIED SCIENCES
VOLUME 15, ISSUE 1: 1026 – 1034 (2025)
Published by: Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria
ISSN: 2315-6856; e-ISSN: 2636-5553; Website: www.jvasonline.com

Evaluation of the phytochemical composition of *Psidium guajava* (guava) methanol leaf extract and the effects of its oral administration on some serum biochemical markers of liver and kidney function in albino rats

Oluchi N. Nwankudu [1] *, Francis N. Agbo [1] and Victoria N. Chiemela [2]

[1] Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

[2.] Department of Agricultural Extension, Faculty of Agriculture, University of Nigeria, Nsukka, Enugu State, Nigeria.

==========

Abstract

Phytochemicals derived from some plants have been shown to possess anti-oxidant, anti-inflammatory, anti-bacterial, anti-cancer and anti-viral activity. Guava (*Psidium guajava*) leaf has been used in ethno-medicine to treat various ailments. This study evaluated the phytochemical constituents of methanol leaf extract of *P. guajava* and the toxicity/safety of the oral administration of the extract to albino rats. Phytochemical analysis of the extract and acute toxicity study followed standard procedures. Sixty albino rats weighing between 100 – 180 g were used for the study. Twenty eight of the rats were used for acute toxicity testing. The remaining 32 rats (made up of 16 males and 16 females) were randomly assigned to four groups. The 16 males were assigned to four groups (M_1, M_2, M_3 and M_4), while the 16 females were also assigned to four groups (F_1, F_2, F_3 and F_4). Groups M_1 and F_1 were the untreated control. Treatment was done through the oral route. The control group in each gender was treated with 2ml/kg body weight of distilled water while the remaining three groups (M_2, M_3 M_4 and F_2, F_3, F_4) were treated with graded doses (200mg/kg, 400mg/kg and 800mg/kg) of the guava leaf extract (GLE), respectively. Treatment lasted for 30 days. At day 31, blood was collected from the rats and the serum derived from the blood sample was used in biochemical assay of liver and kidney function markers and assay of serum lipid profile. Results of the phytochemical analysis showed that *P. guajava* extract contains a high level (+++) of flavonoids and phenols, moderate levels (++) of saponins and alkaloids, and low levels (+) of steroids, terpenoids, glycosides and tannins. Acute toxicity study yielded an oral LD_{50} of 4161.28 mg/kg for the extract in rats. Results of biochemical analysis showed that GLE administration led to significantly ($p < 0.05$) lower serum levels of cholesterol, LDL-C and significantly ($p < 0.05$) high HDL-C in some of the treated groups when compared to the untreated control, but there were no significant effects on serum biochemical markers of liver and kidney function.

Keywords: Phytochemicals analysis; *Psidium guajava*; Guava; Methanol extract; Toxicity/Safety; Liver and Kidney function; Lipid profile.

*** Correspondence:** Oluchi N. Nwankudu; Email: droluchi123@gmail.com; Phone: +2347035294923

Article History: Initial manuscript submission received – Dec. 03, 2024; Final revised form received – May 23, 2025; Accepted for publication – May 31, 2025; Published – June 06, 2025.

Introduction

Guava (*Psidium guajava L.)* is a neotropical plant that belongs to the order of *Myrtales*, family *Myrtaceae,* genus *Psidium*, and species *Psidium guajava* (Arevalo-Marine *et al*., 2021; Hussain *et al*., 2021; Mathiazhagan *et al*., 2023). It is a fast growing evergreen shrub that grows to a height of 3 – 10 metres. It has a shallow root system and the branches are low and drooping. The trunk is usually covered with smooth green to red brown bark that peels off in thin flakes. The leaves grow in pairs and are opposite each other. The leaf blade is 5 – 15 cm long and 3 – 7 cm broad. It is elliptic to oblong in shape. The flowers are solitary or in clusters and are normally white in colour (Asim *et al*., 2022). The plant has a high level of adaptability, which makes it readily available in many parts of the world. Its leaves are used in traditional medicine as well as raw material for pharmaceutical companies.

Ethno-medically, guava leaf in different forms has been reported to be anti-hypertensive (Al-awwadi *et al*., 2004: Ayub *et al*., 2010), cardio-protective (Kumar *et al*., 2021a), anti-oxidant (Quan *et al*., 2019), trypanocidal (Adeyemi *et al*., 2009), anti-bacterial (Sumra *et al*., 2018), anti-viral (Sriwilaijaroen *et al*., 2012) and anti-fungal (Das and Goswani, 2019).

There is paucity of information in available literature on the phytochemical constituents of leaves of *P. guajava* in Eastern Nigeria, and the in vivo safety/toxicity of the leaf extracts, despite its common usage. The present study evaluated the phytochemical constituents of *Psidium guajava* methanol leaf extract and the effects of in vivo oral administration of the extract on serum biochemical markers of liver and kidney function and the serum lipid profile of albino rats.

Materials and Methods

Animals: Sixty albino rats, weighing between 100 – 180 g, were used for the study. They were obtained from the Laboratory Animal Unit of the Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The animals were kept in aluminium cages at room temperature. They were fed with commercial feed and clean drinking water.

Plant material: Fresh guava leaves were harvested early in the morning on the 16th of October, 2021 at Michael Okpara University of Agriculture Umudike, Abia State, Nigeria. They were taken to the Department of Forestry, Michael Okpara University of Agriculture, Umudike, for identification and confirmation as guava leaves. The leaves were washed with clean running water and dried on the laboratory bench for 5 days (Figure 1). The dried leaves were then pulverised. A total of 220 g of the pulverized leaf was subjected to extraction using 98% methanol with intermittent shaking for 48 hours. After the 48 hours, the mixture was filtered. The filtrate was dried using hot air oven at 40°C. The resulting extract was stored at – 4°C in a freezer until needed.

Figure 1. Dried guava (*Psidium guajava*) leaves, used for the study.

Acute toxicity test: Acute toxicity test was conducted using a modified Lorke's two-step method (Lorke, 1983). Twenty-eight albino rats of both sexes were randomly assigned to

seven groups (A – G), of four rats per group. Group A (control) was given 2ml/kg of distilled water, while Groups B, C and D were given 10 mg/kg, 100 mg/kg, 1000 mg/kg of guava leaf extract (GLE) per os, respectively. They were observed for three days for signs of toxicity. When no toxicity was observed in this first batch, 1600 mg/kg, 2900 mg/kg and 5000 mg/kg of GLE was given to groups E, F and G respectively per os. The rats were observed for 72 hours for signs of toxicity and death. The rats were further observed for extra seven days for signs of possible delayed toxicity.

Phytochemical evaluation: Qualitative and quantitative phytochemical evaluation of the guava leaf extract was done according to standard methods described by Pearson (1976) and Harborne (1984), with some modifications as described by other researchers.

In vivo sub-acute toxicity/safety evaluation of the guava leaf extract: A total of 32 albino rats composed of 16 males and 16 females were used for this study. The 16 males were randomly assigned to four groups (M_1, M_2, M_3 and M_4), while the 16 females were also randomly assigned to four groups (F_1, F_2, F_3 and F_4). Groups M_1 and F_1 served as the untreated control, which was given 2 ml/kg of distilled water placebo. Groups M_2 and F_2 were treated with 200 mg/kg of the extract; Groups M_3 and F_3 were treated with 400 mg/kg of the extract; while Groups M_4 and F_4 were treated with 800 mg/kg of the extract. The rats were treated with their group specific doses of the extract via the oral route for 30 days. At day 31, blood for serum biochemistry was collected through the orbital plexus of veins of the median cantus into test tubes, which were left for forty five minutes to clot. The clotted blood was centrifuged at 3000 revolutions per minute to obtain the clear serum supernatant.

Serum biochemical analysis: Serum lipid profile [total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), triglyceride (TG) and high density lipoprotein cholesterol (HDL-C)] assay, was done using a spectrophotometer (752 UV-VIS Spectrometer, RS232, China) and Randox® lipid profile test kits (Randox Laboratories Ltd, Crumlin County Antrim, United Kingdom), and the tests were done according to manufacturer's instruction. Liver function test parameters such as total protein (TP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and bilirubin were assayed using Randox® liver function test kits, but alkaline phosphatase (ALP), urea and creatinine assays were done using Spectrum® test kits (Spectrum Diagnostics, Egypt). The assays were done using colorimeter optimized standard methods according to manufacturer's instruction.

Research Ethics: All the experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of University of Nigeria, Nsukka (Approval Reference No. FVM-IACUC-2020-0262). The procedure approved was also in compliance with Guide for the Care and Use of Laboratory Animals, 8th edition, which upholds recognition and alleviation of pain in laboratory animals according to the Institute for Laboratory Animal Research Publication. We ARRIVE Guidelines for in vivo experimental animal research and reporting, which stresses that the methods used in research and reporting should be easy to replicate, (PLOS BIO. B (6), E1000412 2010) was adhered to.

Statistical analysis: The data collected were analysed using Statistical Package for Social Sciences (SPSS), version 20. Analysis of variance was done and values at ninety-five percent confidence interval ($p < 0.05$) were considered significant. Variant means were separated using Turkey HSD.

Results

Acute toxicity test results: Signs of toxicity were observed in rats given doses of extract 2900 mg/kg and above. Mortality was recorded in the rat group given 5000 mg/kg (Table 1). The calculated LD_{50} was 4161.28 mg/kg.

Phytochemical analysis results: The methanol leaf extract of guava leaf extract used for the study had very high concentration of flavonoids and phenols (Table 2). There were also moderate levels of alkaloids and saponins, as well as low concentration of steroids, terpenoids, glycosides and tannins (Table 2).

Table 1. Result of oral acute toxicity test in rats given varied doses of methanol leaf extract of *Psidium guajava* (guava).

Groups	Dose mg/kg	No. dead/No. in the group	Percentage mortality
A	2ml/kg distilled water	0/4	0%
B	10 mg/kg extract	0/4	0%
C	100 mg/kg extract	0/4	0%
D	1000 mg/kg extract	0/4	0%
E	1600 mg/kg extract	0/4	0%
F	2900 mg/kg extract	0/4	0%
G	5000 mg/kg extract	4/4	100%

Table 2. Phytochemical constituents of methanol leaf extract of *Psidium guajava*.

S/No	Phytochemicals	Qualitative results	Quantitative results (mg/100g)
1	Saponins	++	11.13 ± 0.28
2	Steroids	+	4.60 ± 0.19
3	Flavonoids	+++	28.81 ± 0.95
4	Phenols	+++	39.00 ± 0.31
5	Terpenoids	+	4.13 ± 0.04
6	Glycosides	+	1.96 ± 0.07
7	Alkaloids	++	13.02 ± 0.32
8	Tannins	+	5.82 ± 0.08

Serum lipid profile assay results: Guava leaf extract administration led to significantly ($p < 0.05$) lower serum cholesterol levels in all the extract treated groups, when compared to untreated control (Tables 3a and 3b). The serum HDL-C levels of the females treated with 400 mg/kg and 800 mg/kg GLE were significantly higher than those of others, while that of the males treated with 400 mg/kg was significantly higher than those of other groups (Tables 3a and 3b).. Also, the serum levels of LDL-C of the extract treated rat groups were significantly lower than that of the untreated control (Tables 3a and 3b).

Liver function test results: There were no significant difference between GLE treated rats (both males and females) and the untreated control in serum total protein and bilirubin levels and also serum AST, ALT and ALP activity (Tables 4a and 4b).

Kidney function test results: There was no significant difference between GLE treated rats and the untreated control group in their serum levels of urea and creatinine (Tables 5a and 5b).

Table 3a. Serum lipid profile of female albino rats treated with methanol leaf extract of *Psidium guajava* (MLEPG), compared with an untreated control. [Group F1 = Untreated Control; Group F2 = Treated with 200 mg/kg MLEPG; Group F3 = Treated with 400 mg/kg MLEPG; Group F4 = Treated with 800 mg/kg MLEPG.]

Groups	Total cholesterol (mg/dl)	HDL-C (mg/dl)	Triglycerides (mg/dl)	LDL-C (mg/dl)	VLDL (mg/dl)
Group F1	90.24 ± 8.92 ^{a}	40.85 ± 2.50 ^{a}	107.28 ± 5.98 ^{a}	27.98 ± 11.13 ^{a}	21.41 ± 1.27 ^{a}
Group F2	82.00 ± 2.04 ^{b}	42.89 ± 2.03 ^{ab}	101.50 ± 8.46 ^{ab}	18.81 ± 3.46 ^{ab}	20.30 ± 1.69 ^{a}
Group F3	78.13 ± 1.53 ^{b}	45.41 ± 0.86 ^{b}	103.35 ± 6.78 ^{ab}	12.05 ± 0.99 ^{b}	20.67 ± 1.36 ^{a}
Group F4	75.00 ± 3.93 ^{b}	49.71 ± 3.34 ^{b}	89.41 ± 2.45 ^{b}	7.41 ± 0.81 ^{c}	17.88 ± 0.49 ^{b}

Different alphabetical superscripts in a column indicate significant difference between the groups ($p < 0.05$). [HDL-C = High density lipoprotein cholesterol; LDL-C = Low density lipoprotein cholesterol; VLDL = Very low density lipoprotein.]

Table 3b. Serum lipid profile of male albino rats treated with methanol leaf extract of *Psidium guajava*(MLEPG), compared with an untreated control. [Group M1 = Untreated Control; Group M2 = Treated with 200 mg/kg MLEPG; Group M3 = Treated with 400 mg/kg MLEPG; Group M4 = Treated with 800 mg/kg MLEPG.]

Groups	Total cholesterol (mg/dl)	HDL-C (mg/dl)	Triglycerides (mg/dl)	LDL-C (mg/dl)	VLDL (mg/dl)
Group M1	115.46 ± 19.45 ^{a}	40.46 ± 0.97 ^{a}	115.77 ± 10.14 ^{a}	51.85±21.07 ^{a}	23.16 ± 2.03 ^{a}
Group M2	92.29 ± 13.34 ^{a}	41.57 ± 0.86 ^{a}	115.30 ± 4.50 ^{a}	27.66±12.56 ^{b}	23.06 ± 0.90 ^{a}
Group M3	97.92 ± 5.13 ^{a}	46.91 ± 3.40 ^{b}	97.92 ± 2.56 ^{b}	16.71 ± 9.81 ^{c}	19.58 ± 1.03 ^{b}
Group M4	71.63 ± 6.47 ^{b}	41.59 ± 1.50 ^{a}	111.26 ± 3.12 ^{a}	7.79 ± 6.58 ^{d}	22.25 ± 0.62 ^{a}

Different alphabetical superscripts in a column indicate significant difference between the groups ($p < 0.05$). [HDL-C = High density lipoprotein cholesterol; LDL-C = Low density lipoprotein cholesterol; VLDL = Very low density lipoprotein.]

Table 4a. Liver function biomarker levels in serum of female albino rats treated with methanol leaf extract of *Psidium guajava* (MLEPG), compared with an untreated control. [Group F1 = Untreated Control; Group F2 = Treated with 200 mg/kg MLEPG; Group F3 = Treated with 400 mg/kg MLEPG; Group F4 = Treated with 800 mg/kg MLEPG.]

Groups	Total Proteins (g/dl)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dl)
Group F1	6.24 ± 0.83	67.00 ± 1.83	34.96 ± 1.50	73.43 ± 2.64	0.56 ± 0.06
Group F2	6.29 ± 0.57	64.95 ± 1.26	33.27 ± 2.05	71.15 ± 1.44	0.45 ± 0.06
Group F3	6.60 ± 0.99	65.72 ± 2.37	33.90 ± 2.82	72.11 ± 2.32	0.49 ± 0.08
Group F4	6.03 ± 0.93	63.56 ± 1.70	34.82 ± 1.27	72.00 ± 1.96	0.51 ± 0.02

No significant difference between the groups ($p > 0.05$). [AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; ALP = Alkaline phosphatase.]

Table 4b. Liver function biomarker levels in serum of male albino rats treated with methanol leaf extract of *Psidium guajava* (MLEPG), compared with an untreated control. [Group M1 = Untreated Control; Group M2 = Treated with 200 mg/kg MLEPG; Group M3 = Treated with 400 mg/kg MLEPG; Group M4 = Treated with 800 mg/kg MLEPG.]

Groups	Total Proteins (g/dl)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dl)
Group M1	6.35 ± 0.67	75.23 ± 4.10	37.45 ± 2.62	64.15 ± 2.14	0.48 ± 0.10
Group M2	6.17 ± 0.48	71.64 ± 1.52	34.13 ± 3.18	62.00 ± 1.78	0.43 ± 0.08
Group M3	6.18 ± 0.57	73.80 ± 5.32	35.89 ± 3.26	62.85 ± 2.05	0.47 ± 0.05
Group M4	5.68 ± 0.32	73.73 ± 2.62	33.91 ± 2.75	63.51 ± 1.66	0.49 ± 0.06

No significant difference between the groups ($p > 0.05$). [AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; ALP = Alkaline phosphatase.]

Table 5a. Kidney function biomarker levels in serum of female albino rats treated with methanol leaf extract of *Psidium guajava* (MLEPG), compared with an untreated control. [Group F1 = Untreated Control; Group F2 = Treated with 200 mg/kg MLEPG; Group F3 = Treated with 400 mg/kg MLEPG; Group F4 = Treated with 800 mg/kg MLEPG.]

Parameters	Group F1	Group F2	Group F3	Group F4
Creatinine (mgdl)	0.82 ± 0.08	0.74 ± 0.06	0.79 ± 0.09	0.77 ± 0.08
Urea (mg/dl)	22.21 ± 1.53	20.49 ± 1.37	20.17 ± 2.17	21.38 ± 2.81

No significant difference between the groups ($p > 0.05$).

Table 5b. Kidney function biomarker levels in serum of male albino rats treated with methanol leaf extract of *Psidium guajava* (MLEPG), compared with an untreated control. [Group M1 = Untreated Control; Group M2 = Treated with 200 mg/kg MLEPG; Group M3 = Treated with 400 mg/kg MLEPG; Group M4 = Treated with 800 mg/kg MLEPG.]

Parameters	Group M1	Group M2	Group M3	Group M4
Creatinine (mgdl)	0.92 ± 0.11	0.73 ± 0.05	0.88 ± 0.11	0.78 ± 0.08
Urea (mg/dl)	23.56 ± 2.04	22.57 ± 2.24	21.65 ± 2.47	22.37 ± 1.63

No significant difference between the groups ($p > 0.05$).

Discussion

The toxicity observed in the acute toxicity study at doses higher than 2900 mg/kg and the LD_{50} of 4161.28 mg/kg calculated for the methanol leaf extract of *P. guajava* used for the study suggests that the use of higher doses of the extract for therapy will not be safe. The LD_{50} of 4161.28 mg/kg recorded in the present study for methanol leaf extract of *P. guajava* is different from the > 5000 mg/kg reported for the aqueous leaf extract *P. guajava* by Jaiarj *et al*., (1999) and also the > 5000 mg/kg reported for ethanol leaf extract of *P. guajava* by Ugwuja *et al*. (2022). It is also different from the > 5000 mg/kg reported for methanol bark extract of *P. guajava* by Manekeng *et al*., (2019). It is thought that these differences are due to the chemical nature of the extracting solvent (Kumar *et al*., 2021b) and the plant part being extracted.

The findings in the present study that the guava methanol leaf extract used for the study is rich in flavonoids and phenols, is worthy of note. According to Kumar *et al*. (2021a), flavonoids present in guava leaves determine their antibacterial activity while quercetin which is the most abundant flavonoid in guava leaf extract has anti-diarrheal activities. Moreover, flavonoids have been reported to have anti-oxidant, anti-cancer, anti-viral, anti-inflammatory effects and also preserve neurons and are cardio-protective (Kozlowska and Szostak-Wegierek, 2019). Furthermore, saponins which were found in moderate levels in guava methanol leaf extract is reported to decrease serum lipid proliferation, lower risk of irrational cell multiplication, is anti-diabetic and antidote against heavy metal poisoning (Sharma *et al*., 2023). Also, alkaloids which were found to be in moderate amount in the guava methanol leaf extract used for the study has been reported be antibacterial, anti-mitotic, anti-inflammatory, analgesic and anti-tumor (Debnath *et al*., 2018).

The results of the serum lipid profile assay in the present study which showed that in both females and males, treatment with guava methanol leaf extract led to significantly lower serum cholesterol, LDL-C and triglycerides levels and significantly high HDL-C levels in some of the treated groups suggests that treatment with the extract may protect against atherosclerosis. This is because high LDL-C levels have been reported to be associated with increased risk of heart disease, as LDL-C clogs arteries and leads to atherosclerosis (Soliman, 2019). The findings in the present study on the effects of methanol leaf extract of *P. guajava* concurs with earlier similar reports of the effect of ethanol leaf extract of *P. guajava* on serum lipid profile of albino rats in carbon tetrachloride induced hepatotoxicity (Vijayakumar *et al*., 2018). It also agrees with earlier reports by Tella *et al*. (2019) on the effects of treatment with aqueous leaf extract of *P. guajava* on serum lipid profile of experimentally induced diabetic rats, and a much recent report by Hadi *et al.* (2023) on the effects of leaf extract of *P. guajava* on albino rats fed atherogenic diets.

The finding in the present study that treatment with guava methanol leaf extract led to no significant effects on the serum AST, ALT and ALP activity and the serum levels of creatinine and urea suggests that administration of the extract led to no adverse effects on the liver and kidney, which are vital organs.

Conclusion: The methanol leaf extract of *Psidium gujava* used for the study was rich in flavonoids and phenols, and its administration at the doses used for the study led to significant improvement in the serum lipid profile and no adverse effects on markers of liver and kidney function.

Acknowledgement

We are grateful to our Institutions; Michael Okpara University of Agriculture, Umudike and University of Nigeria, Nsukka, for granting us the privilege to research using our meagre resources.

Competing Interests:

The authors declare no competing interest in this research.

References

Adeyemi OS, Akanji MA, Oguntoye SA. (2009). Ethanolic leaf extract of *Psidium guajava*: phytochemical and trypanocidal activity in rats infected with *Trypanosoma brucei brucei*. *Journal of Medicinal Plants Research*. 3: 420 – 423.

Al-Awwadi NA, Bornet A and Azay J (2004). Red wine polyphenols alone or in association with ethanol prevent hypertension, cardiac hypertrophy, and production of reactive oxygen species in the insulin-resistant fructose fed rat. *Journal of Agriculture and Food Chemistry*, 52: 5593 – 5597.

Arévalo-Marín E, Casas A, Landrum L, Shock MP, Alvarado-Sizzo H, Ruiz-Sanchez E and Clement CR (2021). The taming of *Psidium guajava*: Natural and cultural history of a neotropical fruit. *Frontiers in Plant Science*, 12, 714763. https://doi.org/10.3389/fpls.2021.714763

Asim M, Ullah S, Razzaq A and Quadri S (2022). Varietal discrimination of guava (Psidium guajava) leaves using multi features analysis. *International Journal of Food Properties*, 26(1): 179 – 196.

Ayub MY, Norazmir MN, Mamot S, Jeeven K and Hadijah H (2010). Anti-hypertensive effect of pink guava (*Psidium guajava*) puree on spontentanous hypertensive rats. *International Food Research Journal*, 17: 89 – 96.

Das M. and Goswani S (2019). Antifungal and antibacterial property of guava (Psidium guajava) leaf extract: Role of phytochemicals. *International journal of Health and Sciences and Research*, 9(2): 39 – 45.

Debnath B, Singh WS, Das M, Goswami S, Singh MK, Maiti D. and Manna K. (2018). Role of plant alkaloids on human health: A review of biological activities. Materials Today. *Journal of Chemistry*, 9: 56 – 72.

Hadi NS, Goi M, Wijanarka A, Nuryani K, Setiawan DI, Amalia MR, Domili I, Anasiru MA, Arbie FY, Dewi ABFK and Misnati (2023). Guava (*Psidium guajava*) leaf extract affects lipid profile changes Wistar rats on an atherogenic diet. *Food Research*, 7(Suppl. 5): 54 – 58.

Harborne JB (1984). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. *Springer Nature:* 1 – 141.

Hussain SZ, Naseer B, Qadri T, Fatima T and Bhat TA (2021). Guava (Psidium gujava) – morphology, taxanomy, composition and health benefits. In: Fatima T, Hussain SZ, Qadri T, Bhat TA abd Naseer (Eds.), Fruits Grown in Highland Regions of the Himalayas, pp. 257 – 267.

Jaiarj P, Khoohaswan P, Wongkrajang Y, Peungvicha P, Suriyawong P, Saraya ML and Ruangsomboon O (1999). Anticough and antimicrobial activities of *Psidium guajava* Linn. leaf extract. *Journal of Ethnopharmacology*, *67*(2): 203 – 212.

Kozlowska A. and Szostak-Wegierek D. (2019). Flavonoids-Food sources, Health Benefits, and Mechanisms involved. Nature book. *Bioactive Molecules in Food*, 53 – 78.

Kumar M, Tomar M, Amarowwicz R, Saurabh V, Nair S, Maheshwari C (2021a). Guava (Psidium guajava L.) leaves: nutritional composition, phytochemical profile and health-promoting bioactivities. *Journal of Food,* 10 (4): 752.

Kumar M, Dahuja A, Tiwari S, Punia S, Tak Y, Amarowicz R (2021b). Recent trends in extraction of plant bioactives using green technologies: a review. *Journal of Food Chemistry,* 353: 129441.

Lorke D (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology,* 54: 275 – 287.

Manekeng HT, Mbaveng AT, Ntyam Mendo SA, Agokeng AD and Kuete V (2019). Evaluation of acute and subacute toxicities of *Psidium guajava* methanolic bark extract: A botanical with *in vitro* antiproliferative potential. *Evidence -based Complementary and Alternative Medicine:* eCAM, 2019: 8306986. https://doi.org/10.1155/2019/8306986

Mathiazhagan M, Chinnaiyan V. and Ravishankar KV. (2023). Guava: A natraceutical-rich underutilized fruit crop. In: Kole, C. (eds) Compendium of Crop Genome Designing for Natraceuticals. *Springer Nature*, 1 – 28.

Pearson D. (1976). Chemical Analysis of Foods. 7th Edition, Churchil Livingstone, London.

Quan TH, Benjakul S, Sae-leau T, Balange AK. and Maqsood S (2019). Protein-polyphenol conjugates: Antioxidant property, functionalities and their applications. *Trends in Food Science and Technology,* 91: 507 – 517.

Sharma K, Kaur R, Kumar S, Saini RK, Sharma S, Powde S. and Kumar V (2023). Saponins: A concise review on food related aspects, applications and health implications. *Journal of Food Chemistry Advance,* 2: 100191.

Soliman GA (2019). Dietary fiber, atherosclerosis, and cardiovascular disease. *Nutrients*, 11(5): 1155.

Sriwilaijaroen N, Fukumota S, Kumagai K, Hiramatsu H, Odagiri T, Tashiro M. and Suzuki Y (2012). Antiviral effects of *Psidium guajava Linn*. (Guava) tea on the growth of clinical isolated H1N1 viruses: its role in viral hemagglutination and neutraminidase inhibition. *Journal of Antiviral Responses,* 94 (2): 139 – 146.

Sumra N, Hussain S, Naeem N, Pervaiz M. and Rahman M (2018).The phytochemistry and medicinal value of *Psidium guajava*. *International Journal of Phytomedicine and Phytotherapy,* 4: 32.

Tella, T., Masola, B., & Mukaratirwa, S. (2019). The effect of Psidium guajava aqueous leaf extract on liver glycogen enzymes, hormone sensitive lipase and serum lipid profile in diabetic rats. *Biomedicine & Pharmacotherapy,* 109: 2441 – 2446. https://doi.org/10.1016/j.biopha.2018.11.137

Ugwuja FN, Ezebuiro FC, Omodamiro OD, Ijioma SN and Mukah FE (2022). Antimicrobial activity and anti-diarrheal potentials of *Psidium guajava* Linn leaf extract in experimental rat models. *Animal Research International*, 19(2): 4530 – 4542.

Vijayakumar K, Rengarajan RL, Radhakrishnan R and Anand AV (2018). Hypolipidemic effect of *Psidium guajava* leaf extract against hepatotoxicity in rats. *Pharmacognosy Magazine*, *14*(53): 4 – 8.