



Phytochemical screening and antibacterial activity of leaf and fruit extracts of guava (*Psidium guajava*)

RICHA BHARDWAJ^{1*} and DEVYANI NARUKA¹

IIS (Deemed to be University), Jaipur, Rajasthan 302 020, India

Received: 19 August 2023; Accepted: 18 September 2023

ABSTRACT

An experiment was conducted during 2021–23 at IIS (Deemed to be University) Jaipur, Rajasthan to study the antibacterial efficacy of leaf and fruit extracts of guava (*Psidium guajava* Linn) and to correlate the activity to the phytochemicals present within these plant parts. The study aims to examine primary and secondary metabolites of the extracts qualitatively and quantitatively. The extracts were then used to check the antibacterial efficacy of these plant parts against four bacteria, of which gram-positive were *Bacillus subtilis* (MTCC-441) and *Staphylococcus aureus* (MTCC-737) and gram-negative were *Escherichia coli* (MTCC-739) and *Proteus vulgaris* (MTCC-426). The concentration of total phenols and total flavonoids was higher in leaves than in fruits. The activity of methanolic extracts of leaves higher than that of fruits highest activity, was seen against *E.coli* and lowest activity was seen against *S. aureus*. No activity was recorded against the aqueous extract of both leaf and fruits.

Keywords: Antibacterial, Guava, Methanolic extract, Pathogen, Phytocompounds

Plants are treasure troves of innumerable metabolites endowed within, that actually are responsible for various therapeutic activities. These metabolites include both primary (carbohydrates, lipids, proteins, etc.) and secondary metabolites (phenols, phytosterols, flavonoids, etc.) (Granato *et al.* 2017, Timmis *et al.* 2022), of which secondary metabolites are novel and untapped candidates for intensive pharmacological research so as to combat infectious diseases caused by pathogenic microbes, therefore establishing an underlying reason for the plant to be an infamously traditional medicine (Kumari *et al.* 2017, Mohamed *et al.* 2020, Poddar *et al.* 2020). Guava (*Psidium guajava* Linn) belongs to the family Myrtaceae and is a rich source of compounds that provide it the anti-inflammatory, anti-oxidant, and antimicrobial properties (Joseph *et al.* 2011, Daswani *et al.* 2017, Kafle *et al.* 2018, Donado-Postana *et al.* 2018, Naseer *et al.* 2018). With research and development of new antibiotics to combat newer pathogenic organisms there lies a constant threat of development of Multi-Drug Resistant bacteria. Thus there is an urgent need to find an effective alternative against these MDR organisms. Secondary metabolites such as terpenes and flavonoids extracted from a plant have been shown to have the potential to combat MDR strains, like *B. subtilis*, *E. coli*, *S. pneumoniae*, *B. cereus*, *V. cholerae*, and *S. aureus*, by preventing the synthesis of DNA and

proteins or by rupturing their cell walls (Othman *et al.* 2019, Alvarez-Martinez *et al.* 2021, Khameneh *et al.* 2021).

The present study aims to investigate the presence of primary and secondary metabolites in the leaf and fruit of guava and their antibacterial activity. The study also aims to correlate the metabolites to the antibacterial efficacy of extracts. Qualitative and quantitative analyses were performed to identify and quantify metabolites, and antibacterial activity and MIC of aqueous and methanolic extracts were checked using the agar well diffusion method. Statistical analysis was employed to correlate the appreciable pharmacological activity shown by extracts to constituents present within them.

MATERIALS AND METHODS

The current study was carried out at the Department of Botany, IIS (Deemed to be University) Jaipur, Rajasthan during 2021–23, for which leaves and fruits of guava were collected from the local market of Jaipur, Rajasthan, and identified at herbarium Department of Botany, IIS (Deemed to be University) Jaipur.

Qualitative tests: Primary metabolites like carbohydrates and proteins and secondary metabolites like alkaloids, flavonoids, tannins, glycosides, saponins, and steroids were tested using standard protocols for preliminary phytochemical analysis of plant parts under study (Maharaj *et al.* 2022).

Quantitative tests: The extraction and quantification of total carbohydrates in test extracts were performed

¹IIS (Deemed to be University), Jaipur, Rajasthan.

*Corresponding author email: richa.bhardwaj@iisuniv.ac.in

spectrophotometrically, which included tests for total sugars and starch. For quantification of total soluble sugars (Ext. 1), around 50 mg of each powdered sample was taken and macerated with 20 ml of 80% ethanol using a mortar pestle and then left overnight. Homogenates were centrifuged for 12 min at 1500 rpm, and collected supernatants were then concentrated in a water bath. The volume of concentrate was then raised up to 50 ml with distilled water.

For quantification of starch (Ext. 2), residual pellet obtained from the centrifugation process was collected and suspended in 5 ml of 52% perchloric acid and 6.5 ml of distilled water and shaken continuously for 5 min and centrifuged for 20 min at 2500 rpm. The process was repeated 3 times to collect the supernatant. The volume of the collected supernatant was raised up to 100 ml using distilled water.

For quantification, each test sample (Ext. 1 and 2) was estimated using a phenol-sulphuric acid reagent. A stock solution of glucose (1 mg/ml) was used to prepare the standard regression curve. 1 ml of test sample was taken in test tube and kept in ice-chest. 1 ml of 5% aqueous phenol was added and shaken gently, to this 5 ml of conc. H_2SO_4 was added rapidly and the mixture was allowed to rest in water bath at 25–30°C for 20 min and OD at 490 nm was taken. For the standard curve, different concentration of aq. glucose was taken ($R^2 = 0.991$) (Loomis and Shull 1937, McCreedy *et al.* 1950, Dubois *et al.* 1951).

Lipids-extraction and quantification (Jayaraman 1958): 1 gm of each dried sample powder was homogenize with 10 ml distilled water using a mortar pestle. This homogenate was added in a conical flask with 30 ml chloroform-methanol solution (2:1 v/v) and was left overnight at room temperature, adding 20 ml of chloroform and distilled water. The mixture was transferred into a separating funnel and lower layer containing chloroform with lipids was collected and kept for evaporation in pre-weighed beakers. After evaporation, weight was determined again as the weight of total lipids/g of dried plant material.

*Proteins-extraction and quantification [Lowry *et al.* (1951), Osborne (1962)]:* 60 mg of each dried sample was mixed in 10 ml of cold 10% TCA solution and kept for 30 min and later at 4°C overnight and centrifuged. The supernatant was discarded and residual pellet was suspended in 5% TCA solution and heated at 80°C in water bath for 30 min, later samples were cooled and re-centrifuged and supernatant was discarded. Residual pellet was washed using distilled water and centrifuged again. Residual pellet was collected and dissolved in 10 ml of 1 N NaOH and left overnight at room temperature.

Total protein content was estimated following the method of Lowry *et al.* (1951). 1 ml of each test sample was taken in test tubes and 5 ml of alkaline solution (50 ml of 2% Na_2CO_3 in 0.1 N NaOH and 1 ml of 0.5% $CuSO_4 \cdot 5H_2O$ in Potassium sodium tartrate) was added and kept at room temperature for 10 min. Then, 0.5 ml Folin-Ciocalteu reagent was added to each test tube and mixed thoroughly. After 30 min, OD was taken at 750 nm using

a spectrophotometer. For the standard regression curve, BSA solution (1 mg/ml in 1 N NaOH) ($R^2 = 0.994$), was taken at different concentrations and made up to 1 ml using distilled water. The test was run in triplicates and the mean value was taken.

Ascorbic acid- extraction and quantification (Roe and Kuenther 1943): Each fresh test material (400 mg) was homogenized with 10 ml of acetate buffer (pH 4.8) and centrifuged at 1500 rpm for 15 min. To 1 ml of supernatant, separately collected and measured 4 ml of 4% TCA was added, left overnight, and centrifuged. To the supernatant of each sample, 1 ml of the colour reagent (90 ml of 2.2%, 2,4-dinitrophenylhydrazine in 10 N H_2SO_4 , 5 ml of 5% thiourea and 5 ml of 0.6% $CuSO_4$ solution), was added and incubated at 57°C for 45 min. Later, 7 ml of 65% H_2SO_4 was added to each tube and cooled again.

For preparation of the standard curve, the stock solution of ascorbic acid (10 mg/100 ml in 4% TCA), and varied concentrations (0.01 to 0.09 mg/ml) ($R^2 = 0.991$) were prepared. To each tube, 4% TCA solution was added to raise the volume up to 5 ml and kept overnight at room temperature. To these 1 ml of the colour reagent was added. 7 ml of 65% H_2SO_4 was added to each tube later and ODs were measured at 540 nm.

*Alkaloids (Oncho *et al.* 2021):* Alkaloids content was estimated using 3 gm of each sample dried powder mixed with 20 ml of 10% acetic acid in ethanol in a flask, covered and kept for 4 h. Later solution was filtered and concentrated ammonium hydroxide was added drop by drop to the filtrate until no ppt formation was observed. The solution was kept to settle down the precipitate and later, the precipitate was collected and washed with 10% ammonium hydroxide and filtered. Filtered precipitate was kept for drying in a pre-weighed petri dish and weight was again measured after drying. Alkaloid content was calculated as mg/gm of sample powder used.

*Total phenolic content assay [McDonald *et al.* (2001), Ebrahimzadeh *et al.* (2008), Nabavi *et al.* (2008), Bhardwaj *et al.* (2014)]:* Total phenolic content was estimated by the Folin-Ciocalteu method using methanolic leaf and fruit extract. 0.5 ml of each extracted sample (1 mg/ml) was taken in test tubes and diluted 10 times using methanol. Then 5 ml Folin-Ciocalteu reagent (1:10 diluted using distilled water) was added and mixed. 5 min later, 4 ml of 1 M aq. Na_2CO_3 was added and the mixture was allowed to rest for 15 min. OD was taken at 765 nm using spectrophotometer. For standard curve preparation, Gallic acid (1 mg/ml in methanol) was used at different concentrations (50–300 $\mu g/ml$) ($R^2 = 0.996$). Total phenol values are expressed in terms of Gallic acid equivalent (mg/g of dry mass).

*Total flavonoid content assay [Dewanto *et al.* (2002), Sakanaka *et al.* (2005), Bhardwaj *et al.* (2014)]:* Total flavonoid content was determined by using the aluminium chloride colorimetric method ($AlCl_3$) using quercetin as standard. 1 ml of test material was added to the test tube and 4 ml of distilled water was added. To above mixture, 0.3 ml of 5% $NaNO_2$ was added. After 5 min, 0.3 ml of

10% AlCl_3 was added. Then 6 min later, 2 ml of 1 M NaOH was added, and total volume was raised up to 10 ml with distilled water. Absorbance was measured against a blank at 510 nm using a spectrophotometer. The standard curve was prepared using the solution of Quercetin (1 mg/ml in methanol) in different concentrations (0.5–5.0 mg/ml) ($R^2 = 0.991$). Total flavonoid content of the extracts was expressed in mg of quercetin equivalents/gdw.

Antibacterial assay and MIC

Soxhlet extraction: Extracts were prepared using 20 gm of dried fruit and leaf powder each with 200 ml of methanol separately. Oven-dried samples for each aqueous and methanolic extracts were prepared.

Well-diffusion method was employed for antibacterial assay of aqueous and methanolic extracts of leaf and fruit (Sen and Batra 2012). Gram positive- *Bacillus subtilis* (MTCC-441), *Staphylococcus aureus* (MTCC-737) and Gram negative- *Escherichia coli* (MTCC-739), *Proteus vulgaris* (MTCC-426) were used for assay. Bacteria were revived in nutrient broth using an incubator shaker (16–24 h). 150 μl of bacterial culture from broth was spread over nutrient agar plates and kept for 20 min for better absorption. Then 30 μl of test sample was poured in well punched. To calculate MIC, methanolic and aqueous leaf and fruit extract of different concentrations (100 mg/ml and 500 mg/ml) were poured into wells along with positive and negative controls (30 μl each) [Streptomycin (1 mg/ml) was used as a positive control]. Plates were sealed using paraffin to avoid any contamination and kept in an incubator at 37°C for 16–24 h after which the inhibition zone was measured.

Statistical analysis: For correlating the plant metabolites and their activities to the profound antimicrobial activity t-test and regression analysis were used.

RESULTS AND DISCUSSION

Table 1 indicates the results of qualitative tests for the estimation of the presence of primary and secondary plant metabolites in the leaf and fruit of guava. Leaf extract showed the presence of all tested metabolites except Saponins. Results were similar to the study done by Kaneria *et al.* (2011) and Dubale *et al.* (2023).

For quantitative estimation, plant metabolite contents were analyzed in mg/g dry weight of the test sample (Table 2). Total soluble sugar content was recorded more in fruit extract (6.4 ± 0.006 mg/gdw) than in leaf extract (5.0 ± 0.003 mg/gdw). Similarly, fruit extract had more soluble starch content than leaf extract (Fruit extract, 4.14 ± 0.019 mg/gdw; Leaf extract, 3.96 ± 0.017 mg/gdw). Fruit extract (3.83 ± 0.016 mg/gdw) showed the presence of more protein content than leaf extract (1.0 ± 0.012 mg/gdw). Lipid content was observed more in leaf (73.3 ± 4.2 mg/gm of leaf powder) than fruit (16.2 ± 1.07 mg/gm of fruit powder). Ascorbic acid content was recorded more in leaf (0.225 ± 0.006 mg/gdw) than in fruit (0.183 ± 0.007 mg/gdw). Leaf extract (14.3 ± 2.3 mg/gm) showed more alkaloid content than fruit extract (5 ± 1.01 mg/gm). The result of

Table 1 Qualitative analysis of primary and secondary metabolites of guava leaf and fruit

Plant metabolites	Procedure	Observation	Leaf	Fruit
Carbohydrates	Molisch's test	Blackish-purple ring	+	+
	Fehling's test	Orange-brick reddish ppt	+	+
Protein	Ninhydrin test	Blue colour	+	+
	Xanthoproteic test	Yellow colour	+	+
Alkaloids	Mayer's test	White or pale ppt	+	+
Flavonoids	Alkaline reagent test	Yellow colour	+	+
	Shinoda's test	Orange-red colour	+	+
Tannins	Lead acetate test	Yellow or red ppt	+	+
Phenolics	Ferric chloride test	Blue-green colour	+	+
Glycosides	Glycosides test	Yellowish white ppt	+	+
Lignin	Wiesner's test	Red ppt	+	+
Saponins	Foam test	Foam	+	-
Steroids	Salkowski's test	Yellow-orange colour	+	+

+ = compound present; - = compound absent

quantitative estimation is in accordance with the results of the study done by Daniel and Krishnakumar (2015). Total phenolic content (TPC) and total flavonoid content (TFC) were estimated in mg gallic acid equivalent per gram dry weight of the sample (mg GAE/gdw) and mg quercetin equivalent per gram dry weight of sample (mg QE/gdw) respectively. Both TPC and TFC were recorded more in leaf extract than in fruit extract. TPC of leaf extract was 70.05 ± 0.003 mg GAE/gdw and fruit extract was 49.38 ± 0.005 mg GAE/gdw. TFC of leaf extract was 2.76 ± 0.014 mg QE/gdw and fruit extract was 0.69 ± 0.007 mg QE/gdw. The result of TPC and TFC analysis are closely similar to the study done by Kaneria *et al.* (2011), Gorse *et al.* (2022) and Pandhi *et al.* (2022).

Antibacterial test: Results from the antibacterial assay showed that aqueous extracts of leaf and fruit were nonresponsive against all 4 bacteria under test (Table 3). At higher concentrations, both leaf and fruit methanolic extracts showed promising antibacterial activity against all 4 pathogenic bacteria. MIC for methanolic fruit extract was at 75 mg/ml against *P. vulgaris* while against *B. subtilis* and *E. coli* in methanolic leaf extract was at 25 mg/ml. Test samples showed maximum inhibition against *Bacillus subtilis* while minimum against *P. vulgaris*.

A comprehensive study of recent research suggests that the majority of bioactive components isolated from plant parts demonstrate an efficient antibacterial activity against

Table 2 Quantitative estimation of plant metabolites in leaf and fruit of guava

Sample	Quantitative estimation of plant metabolites							
	Carbohydrates (mg/gdw)		Protein	Lipids	Ascorbic acid	Alkaloids	TPC	TFC
	Soluble sugars	Soluble starch	(mg/gdw)	(mg/gdw)	(mg/gdw)	(mg/gdw)	(GAE/gdw)	(QE/gdw)
Leaf	5.0±0.003	3.96±0.017	1.0±0.012	73.3±4.2	0.225±0.006	14.3±2.3	70.05 ± 0.003	2.76 ± 0.014
Fruit	6.4±0.006	4.14±0.019	3.83±0.016	16.2±1.07	0.183±0.007	5.0±1.01	49.38 ± 0.005	0.69 ± 0.007

TPC, Total phenolic content; TFC, Total flavonoid content.

Table 3 Antibacterial activity of leaf and fruit extracts against different bacteria

Microorganism Bacteria		Methanol				Aqueous			
		Leaf		Fruit		Leaf		Fruit	
		100 mg/ml	500 mg/ml	100 mg/ml	500 mg/ml	100 mg/ml	500 mg/ml	100 mg/ml	500 mg/ml
<i>Bacillus subtilis</i> (MTCC-441)	IZ	10.66±1.25	11.66±0.47	5.5±0.50	8.67±0.47	0	0	0	0
	AI	0.46	0.47	0.24	0.35	-	-	-	-
<i>Escherichia coli</i> (MTCC-739)	IZ	13±1.63	12±4.08	5.67±0.90	11.33±3.29	0	0	0	0
	AI	0.55	0.50	0.24	0.48	-	-	-	-
<i>Proteus vulgaris</i> (MTCC-426)	IZ	11.33±1.7	12.66±2.86	8.0±0.80	12±3.74	0	0	0	0
	AI	0.47	0.54	0.33	0.51	-	-	-	-
<i>Staphylococcus aureus</i> (MTCC-737)	IZ	12±2.1	11.33±2.86	6.67±2.05	8.0±2.16	0	0	0	0
	AI	0.48	0.46	0.27	0.34	-	-	-	-

IZ, inhibition zone; AI, activity index.

the pathogens responsible for various infectious diseases and skin-related problems. Phenolic compounds are identified as principal compounds crucial for various pharmacological activities, these activities may be attributed to the structure i.e. p-hydroxy benzoic acid several functional groups with ester side chains, flavonoids with the basic skeleton structure of phenols, are polyphenolic compounds with flavan nucleus also competent against pathogens by disrupting their cell wall and degrading their genetic material thus challenging conventional modes of treatment (Ecevit *et al.* 2022, Lobiuc *et al.* 2023). The above study concludes and substantiates the literature survey, statistically proving the efficacy of methanolic extracts of leaves which are also shown to have higher amounts of TPC and TFC against the pathogenic Gram-positive and Gram-negative bacteria. (Aghraz A *et al.* 2020, Soleimani *et al.* 2022). The lowest MIC of methanolic extract was 25 mg/ml against *B.subtilis* and *E.coli* and 75 mg/ml against *P. vulgaris*. This therefore suggests a low amount of active ingredient is enough to inhibit these pathogens. Results of the t-test and linear regression analysis suggest that there is a strong correlation between the metabolites in each extract and their antimicrobial activity. The p-value for the F-statistic is less than the chosen significance level 0.05, thus we conclude that the regression model, as a whole, is statistically significant. The study thus concludes that guava is rich in bioactive compounds which are responsible for the antimicrobial activity exhibited by the test extracts and hence forms a competent contender for pharmacological uses.

REFERENCES

Alvarez-Martinez F J, Barrajon-Catalan E, Herranz-Lopez M and

Micol V. 2021. Antibacterial plant compounds, extracts and essential oils: An updated review on their effects and putative mechanisms of action. *Phytomedicine* **90**: 153626.

- Aghraz A, Albergamo A, Benameur Q, Salvo A, Larhsini M, Markouk M, Gervasi T and Cicero N. 2020. Polyphenols contents, heavy metals analysis and in vitro antibacterial activity of extracts from *Cladanthus arabicus* and *Bubonium imbricatum* of Moroccan Origin. *Natural Product Research* **34**(1): 63–70.
- Bhardwaj R, Yadav A and Sharma R. 2014. Phytochemicals and antioxidant activity in *Boerhavia diffusa*. *International Journal of Pharmacy and Pharmaceutical Sciences* **6**(1): 344–48.
- Daniel G and Krishnakumari S. 2015. Quantitative analysis of primary and secondary metabolites in aqueous hot extract of *Eugenia uniflora* (L) leaves. *Asian Journal of Pharmaceutical and Clinical Research* **8**(1): 334–38.
- Daswani P G, Gholkar M S and Birdi T J. 2017. *Psidium guajava*: A single plant for multiple health problems of rural Indian population. *Pharmacognosy reviews* **11**(22): 167–74.
- Dewanto V, Wu X, Adom K K and Liu R H. 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry* **50**: 3010–14.
- Donado-Pestana C M, Moura M H C, de Araujo R L, de Lima Santiago G, de Moraes Barros H R and Genovesi M I. 2018. Polyphenols from Brazilian native Myrtaceae fruits and their potential health benefits against obesity and its associated complications. *Current opinion in food science* **19**: 42–49.
- Dubale S, Kebebe D, Zeynudin A, Abdissa N and Suleman S. 2023. Phytochemical screening and antimicrobial activity evaluation of selected medicinal plants in Ethiopia. *Journal of experimental pharmacology* **15**: 51–62.
- Dubois M, Gilles K, Hamilton J K, Rebers P A and Smith F. 1951. A colorimetric method for the determination of sugar. *Nature* **168**: 167.

- Ebrahimzadeh M A, Pourmorad F and Bekhradnia A R. 2008. Chelating activity screening, phenol and flavonoid content of some medicinal plants from Iran. *African Journal of Biotechnology* **32**: 43–49.
- Ecevit K, Barros A A, Silva J M and Reis R L. 2022. Preventing microbial infections with natural phenolic compounds. *Future Pharmacology* **2**(4): 460–98.
- Gorse M K, Bele P and Udaipure S K. 2022. Phytochemical screening and quantitative analysis of active phytochemicals of *Guizotia abyssinica* seed to know of their therapeutic values. *International Journal of Health Sciences* **6**(S2): 2880–92.
- Granato D, Nunes D S and Barba F J. 2017. An integrated strategy between food chemistry, biology, nutrition, pharmacology, and statistics in the development of functional foods: A proposal. *Trends in Food Science and Technology* **62**: 13–22.
- Jayaraman J. 1958. *Laboratory Manual in Biochemistry*, pp. 96–97. Wiley Eastern Limited, New Delhi.
- Joseph B and Priya M. 2011. Review on nutritional, medicinal and pharmacological properties of guava (*Psidium guajava* Linn.). *International Journal of Pharma and Biosciences* **2**(1): 53–69.
- Kafle A, Mohapatra S S, Reddy I and Chapagain M. 2018. A review on medicinal properties of *Psidium guajava*. *Journal of Medicinal Plants Studies* **6**(4): 44–47.
- Kaneria M and Chanda S. 2011. Phytochemical and pharmacognostic evaluation of leaves of *Psidium guajava* L. (Myrtaceae). *Pharmacognosy Journal* **3**(23): 41–45.
- Khameneh B, Eskin N A M, Iranshahy M and Fazly Bazzaz B S. 2021. Phytochemicals: A promising weapon in the arsenal against antibiotic-resistant bacteria. *Antibiotics (Basel, Switzerland)* **10**(9): 1044.
- Kumari P, Kumari C and Singh P S. 2017. Phytochemical screening of selected medicinal plants for secondary metabolites. *International Journal of Life Sciences and Scientific Research* **3**(4): 1151–57.
- Lobiuc A, Paval N-E, Mangalagiu II, Gheorghita R, Teliban G-C, Amariuca-Mantu D and Stoleru V. 2023. Future antimicrobials: Natural and functionalized phenolics. *Molecules* **28**(3): 1114.
- Loomis W E and Shull C A. 1937. *Methods in Plant Physiology*. McGraw-Hill Book Co. New York.
- Lowry O H, Rosebrough N J, Farr A L and Randall R J. 1951. Protein measurement with the Folin-phenol reagent. *Journal of Biological Chemistry* **193**: 265–75.
- Maharaj A, Naidoo Y, Dewir Y H and Rihan H. 2022. Phytochemical screening, and antibacterial and antioxidant activities of *Mangifera indica* L. leaves. *Horticulturae* **8**(10): 909.
- McCready R M, Guggoiz J, Silveira V and Owens H S. 1950. Determination of starch and amylase in vegetables. *Analytical Chemistry* **22**: 1156–58.
- McDonald S, Prenzler P D, Autolovich M and Robards K. 2001. Phenolic content and antioxidant activity of olive extracts. *Food Chemistry* **73**: 73–84.
- Mohamed E A A, Muddathir A M and Osman M A. 2020. Antimicrobial activity, phytochemical screening of crude extracts, and essential oils constituents of two *Pulicaria* spp. growing in Sudan. *Scientific Reports* **10**: 17148.
- Nabavi S M, Ebrahimzadeh M A, Nabavi S F, Hamidinia A and Bekhradnia A R. 2008. Determination of antioxidant activity, phenol and flavonoids content of *Parrotia persica* Mey. *Pharmacologyonline* **2**: 560–67.
- Naseer S, Hussain S, Naeem N, Pervaiz M and Rahman M. 2018. The phytochemistry and medicinal value of *Psidium guajava* (guava). *Clinical phytoscience* **4**(1): 1–8.
- Oncho D A, Ejigu M C and Urgessa O E. 2021. Phytochemical constituent and antimicrobial properties of guava extracts of east Hararghe of Oromia, Ethiopia. *Clinical Phytoscience* **7**(1): 1–10.
- Osborne D J. 1962. Effect of kinetin on protein and nucleic acid metabolism in *Xanthium* leaves during senescence. *Plant Physiology* **37**: 595–602.
- Othman L, Sleiman A and Abdel-Massih R M. 2019. Antimicrobial activity of polyphenols and alkaloids in middle eastern plants. *Frontiers in Microbiology* **10**: 911.
- Pandhi S, Kumar A and Rai D C. 2022. Efficacy evaluation of extraction technologies for guava (*Psidium guajava* L.) leaves extract. *Annals of Phytomedicine* **11**(1): 413–18.
- Poddar S, Sarkar T, Choudhury S, Chatterjee S and Ghosh P. 2020. Indian traditional medicinal plants: A concise review. *International Journal of Botany Studies* **5**(5): 174–90.
- Roe J H and Kuenther C A. 1943. The determination of ascorbic acid in whole blood and urine through the 2, 4-nitrophenylhydrazine derivative. *Journal of Biological Chemistry* **147**: 399–407.
- Sakanaka S, Tachibana Y and Okada Y. 2005. Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). *Food Chemistry* **89**: 569–75.
- Sen A and Batra A. 2012. Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: *Melia azedarach* L. *International Journal of Current Pharmaceutical Research* **4**(2): 67–73.
- Soleimani M, Arzani A, Arzani V and Roberts T H. 2022. Phenolic compounds and antimicrobial properties of mint and thyme. *Journal of Herbal Medicine* **36**: 100604.
- Timmis A, Vardas P, Townsend N, Torbica A, Katus H, De Smedt D and Achenbach S. 2022. European society of cardiology: Cardiovascular disease statistics 2021. *European Heart Journal* **43**(8): 716–99.