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Evaluation and Quantification of Flavonoids and Terpenoids from Plant Leaves by HPLC Method.

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ABSTRACT

This study explores bioactive compounds in plants, focusing on flavonoids and azadirachtin, known for their antioxidant, anti-inflammatory, and therapeutic properties. Flavonoids such as Morin, Naringin, Quercetin, and Rutin—are analyzed in the leaves of *Psidium guajava* (Guava), *Pongamia pinnata* (Pongamia), *Rubus fruticosus* (Blackberry), and *Mangifera indica* (Mango). Whereas Azadirachtin, a tetranortriterpenoid with biopesticidal and medicinal benefits, is assessed in the leaves of *Azadirachta indica* (Neem). High-Performance Liquid Chromatography (HPLC) with a reverse-phase column is used for qualitative and quantitative analysis. The mobile phase used for flavonoids is 0.1% phosphoric acid and acetonitrile (1:3) whereas the mobile phase used for azadirachtin is methanol and water (3:2). This study identified flavonoids and azadirachtin in above plant leaves, with specific retention times (RT) confirming their presence. In *Psidium guajava* (Guava), Naringin and Quercetin were detected at 7.13 min and 2.527 min, respectively. *Pongamia pinnata* (Pongamia) contained Morin, Naringin, Quercetin, and Rutin at 3.887 min, 7.130 min, 2.527 min, and 4.780 min, respectively. *Rubus fruticosus* (Blackberry) exhibited Quercetin, Morin, and Naringin at 2.567 min, 3.887 min, and 5.673 min, respectively, while *Mangifera indica* (Mango) showed the presence of Quercetin and Morin at 2.593 min and 3.393 min, respectively. For azadirachtin in *Azadirachta indica* (Neem), concentrations and RTs varied by leaf condition: dried (5.85 µg/mL, 3.327 min), semi-dried (20.12 µg/mL, 3.253 min), and fresh (11.71 µg/mL, 2.305 min). obtained results concluded that quercetin is a more prominent flavonoid present in tested plant leaves whereas remaining flavonoids exist in lower quantities. Azadirachta shown a significant quantity in neem leaves but the previous studies conclude that it is more prominent in neem cornels. This study extends to the medicinal and remedial studies of various diseases and their physiological effects.

Keywords: Bioactive compounds, flavonoids, azadirachtin, High-Performance Liquid Chromatography (HPLC), antioxidant properties.

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INTRODUCTION

Mangifera indica (Mango), *Rubus fruticosus* (Blackberry), *Pongamia pinnata* (Pongamia), *Psidium guajava* (Guava), and *Azadirachta indica* (Neem) are significant plants with diverse applications in nutrition, medicine, ecology, and industry. Mango is a highly valued fruit, rich in vitamins A and C, carotenoids, and flavonoids, making it beneficial for immunity and digestion [1]. It also has strong antioxidant and anti-inflammatory properties, supporting cardiovascular and skin health [2]. Blackberry is equally rich in bioactive compounds, particularly anthocyanins and flavonoids, which contribute to its strong antioxidant and antidiabetic properties [3]. It is commonly used in dietary supplements, jams, and wines, playing a crucial role in gut health and cardiovascular protection [4].

Pongamia, in contrast, is primarily valued for its biofuel potential. Its seeds contain karanjin and pongamol, compounds with medicinal and pesticidal properties. Additionally, *Pongamia pinnata* is a nitrogen-fixing tree, improving soil fertility and making it an excellent choice for reforestation and agroforestry [5]. Guava is a highly nutritious fruit, known for its exceptional vitamin C content and antimicrobial properties [6]. It is widely used in the food and pharmaceutical industries, with studies supporting its role in diabetes management and digestion improvement [7]. The leaves of guava also contain quercetin and other flavonoids, which contribute to their antibacterial effects [8].

Neem is a powerhouse of medicinal applications due to its rich composition of azadirachtin, Nimbin, and flavonoids, making it an effective natural pesticide, antimicrobial, and antifungal agent [9]. It plays a significant role in sustainable agriculture by acting as a bio-pesticide, reducing reliance on synthetic chemicals [10]. Neem is also used extensively in pharmaceuticals, cosmetics, and traditional medicine due to its wound-healing and immune-boosting properties [11]. Ecologically, neem and *Pongamia pinnata* are crucial for environmental sustainability, contributing to reforestation, soil conservation, and pest management [12].

Whereas mango, blackberry, and guava are predominantly valued for their nutritional and medicinal benefits, pongamia and neem hold immense industrial and ecological significance. All five plants share high flavonoid content, contributing to their strong medicinal properties [13]. Their combined significance underscores their importance in human health, agriculture, and environmental conservation, making them valuable across multiple domains.

Table 1: Taxonomy of Mango, Blackberry, Pongamia, Guava, and Neem.

Feature	Mango (<i>Mangifera indica</i>)	Blackberry (<i>Rubus fruticosus</i>)	Pongamia (<i>Pongamia pinnata</i>)	Guava (<i>Psidium guajava</i>)	Neem (<i>Azadirachta indica</i>)
Kingdom	Plantae	Plantae	Plantae	Plantae	Plantae
Phylum	Angiosperms	Angiosperms	Angiosperms	Angiosperms	Angiosperms
Class	Eudicots	Eudicots	Eudicots	Eudicots	Eudicots
Order	Sapindales	Rosales	Fabales	Myrtales	Sapindales
Family	Anacardiaceae	Rosaceae	Fabaceae	Myrtaceae	Meliaceae
Genus	<i>Mangifera</i>	<i>Rubus</i>	<i>Pongamia</i>	<i>Psidium</i>	<i>Azadirachta</i>
Species	<i>Mangifera indica</i>	<i>Rubus fruticosus</i>	<i>Pongamia pinnata</i>	<i>Psidium guajava</i>	<i>Azadirachta indica</i>

Flavonoids and terpenoids are vital phytochemicals with extensive applications in medicine, agriculture, and human health. Among flavonoids, morin, naringin, quercetin, and rutin are known for their strong antioxidant, anti-inflammatory, and therapeutic properties. Morin and rutin are found in various fruits such as guava, onion, and other fruits, which exhibit potential antioxidant, anticancer effects, neuroprotective, and cardioprotective benefits too [1]. Naringin, abundant in citrus fruits, is known for its anti-inflammatory, antiviral, and cholesterol-lowering properties, making it significant in cardiovascular health and metabolic disorders [14]. Quercetin, one of the most widely studied flavonoids, is present in onions, berries, and apples. It is key in reducing oxidative stress, supporting immune function, and offering anticancer and neuroprotective effects [13]. Rutin, structurally related to quercetin and found in guava, blackberries, and citrus fruits, is particularly known for its vascular health benefits, strengthening capillaries and reducing the risk of varicose veins and cardiovascular diseases [3].

In contrast to flavonoids, azadirachtin is a terpenoids derived from neem (*Azadirachta indica*) and is primarily known for its role in pest control. Unlike flavonoids, which focus on human health benefits, azadirachtin is an ecological and agricultural asset due to its potent insecticidal, antifungal, and antimicrobial properties [9]. It disrupts insect growth and reproduction, making neem-based pesticides a sustainable alternative to synthetic chemicals [10]. Additionally, azadirachtin has applications in pharmaceuticals for its anti-inflammatory and immune-boosting properties [11].

While flavonoids like morin, naringin, quercetin, and rutin primarily function as antioxidants, cardio protective agents, and immune boosters, azadirachtin serves as an environmental protector, bio-pesticide, and medicinal compound [15]. The convergence of these phytochemicals in medicine, nutrition, and agriculture highlights their significance in human health and sustainability.

Table 2: Comparative Significance of Mango, Blackberry, Pongamia, Guava, and Neem.

Aspect	Mango (<i>Mangifera indica</i>)	Blackberry (<i>Rubus fruticosus</i>)	Pongamia (<i>Pongamia pinnata</i>)	Guava (<i>Psidium guajava</i>)	Neem (<i>Azadirachta indica</i>)
Medicinal Uses	Contains polyphenols, flavonoids, and carotenoids with antioxidant, anti-inflammatory, and anticancer properties.	Rich in anthocyanins, flavonoids, and vitamin C, useful in managing diabetes and improving heart health.	Leaves and seeds contain bioactive compounds for treating skin diseases, ulcers, and inflammation.	High vitamin C content, antibacterial and antidiabetic properties.	Contains azadirachtin, nimbin, and flavonoids, with strong antimicrobial and antifungal effects.
Nutritional Value	High in vitamin C, vitamin A, and fiber; supports immunity and digestion.	Rich in vitamin C, fiber, and antioxidants, beneficial for gut health.	Seeds contain fatty acids and proteins; not commonly consumed but used in medicine.	Contains vitamin C, lycopene, and dietary fiber, beneficial for digestion and immunity.	Not consumed directly but neem oil has medicinal applications.
Ecological Role	Important for pollinators and provides shade.	Supports wildlife by providing food for birds and mammals.	Acts as a nitrogen fixer, improving soil fertility.	Attracts pollinators and supports agroforestry.	Helps in reforestation and acts as a natural pesticide.
Industrial Uses	Used in food, cosmetics, and traditional medicine.	Utilized in jams, wines, and dietary supplements.	Produces biofuel from seeds, also used in pharmaceuticals.	Used in food, cosmetics, and pharmaceuticals.	Used in pest control, cosmetics, pharmaceuticals, and agriculture.
Phytochemical Content	Contains mangiferin, quercetin, and carotenoids.	High in anthocyanins, flavonoids, and ellagic acid.	Rich in pongamol, karanjin, and flavonoids.	Contains quercetin, lycopene, and gallic acid.	Contains azadirachtin, nimbin, and flavonoids.
Environmental Benefits	Acts as a carbon sink and improves air quality.	Aids in erosion control and supports biodiversity.	Improves soil health by nitrogen fixation.	Helps prevent soil erosion and maintains biodiversity.	Drought-resistant and provides natural pest control.



Based on the above studies, the current research focused on to evaluate and quantify flavonoids such as Morin, Naringin, Quercetin, and Rutin—along with the terpenoid Azadirachtin found in Neem (*Azadirachta indica*). High-Performance Liquid Chromatography (HPLC) is used for the precise isolation and quantification of the compounds. The specific objectives are, extraction and isolation of the flavonoids (leaves of Mango, Blackberry, Guava, and Pongamia) and terpenoids from the Neem plant leaves by using HPLC technique, as well a comparative study on the analysis the flavonoids and terpenoids present in the plant leaves.

METHODOLOGY

The evaluation of flavonoids and terpenoids in *Mangifera indica* (Mango), *Psidium guajava* (Guava), *Rubus fruticosus* (Blackberry), *Pongamia pinnata* (Pongamia), and *Azadirachta indica* (Neem) involves a systematic methodology encompassing shade drying of leaves, Soxhlet extraction, and High-Performance Liquid Chromatography (HPLC) analysis. This approach ensures the efficient isolation and quantification of bioactive compounds [16].

Preparation and Extraction of Plant Extract

Leaf samples, including both newborn and mature leaves, are collected. The leaves are dried using the shade-dry method, then crushed using a mortar and pestle until a fine powder is obtained. The powder is stored in a dark environment until further use [17].

Soxhlet Extraction

Samples are weighed (20 grams) and prepared for extraction. The Soxhlet apparatus is cleaned using ethanol before loading the sample and solvent system. The setup is maintained at a heating temperature of 30°C, and eight extraction cycles are performed. The resulting extract is stored at room temperature for further analysis [18].

High-Performance Liquid Chromatography (HPLC)

High-Performance Liquid Chromatography (HPLC) is used to separate, identify, and quantify compounds in the plant extracts. The mobile phase consists of a mixture of water, organic solvents, and buffers at a specific pH. The sample is dissolved in a suitable solvent and filtered through a 0.22 or 0.45 µm filter to remove particulates. The HPLC system includes a pump, injector, column, and detector. The mobile phase is pumped through the column at a constant flow rate, and the sample is injected into the system using an autosampler or manual injector. Separation occurs based on interactions with the stationary phase, and detection is achieved using a UV-Vis detector. Retention times of the compounds are recorded, and data analysis is performed to determine the concentration and purity of the analytes based on peak areas or heights. The column is flushed with a high proportion of organic solvent post-analysis to remove residual compounds and maintain performance [19].

RESULTS

The HPLC analysis revealed significant variations in the retention times and concentrations of flavonoids and azadirachtin across different plant samples. The standard HPLC method for flavonoids was conducted using an RP column with a mobile phase of 0.1% H₃PO₄ and acetonitrile (25:75), a flow rate of 1 mL/min, a detection wavelength of 350 nm, and a column temperature of 37°C. The retention times for the standard flavonoids were recorded as follows: morin at 3.887 min, naringin at 7.130 min, quercetin at 2.513 min, and rutin at 4.780 min as per standard chromatograms (Figures 1–4). In comparison, the analysis of mango leaf extract showed similar retention patterns, with quercetin detected at 2.593 min (peak area: 57.1761 mAUmin) and morin at 3.393 min (peak area: 201.3511 mAUmin), indicating a higher concentration of morin compared to quercetin (Figure 5).

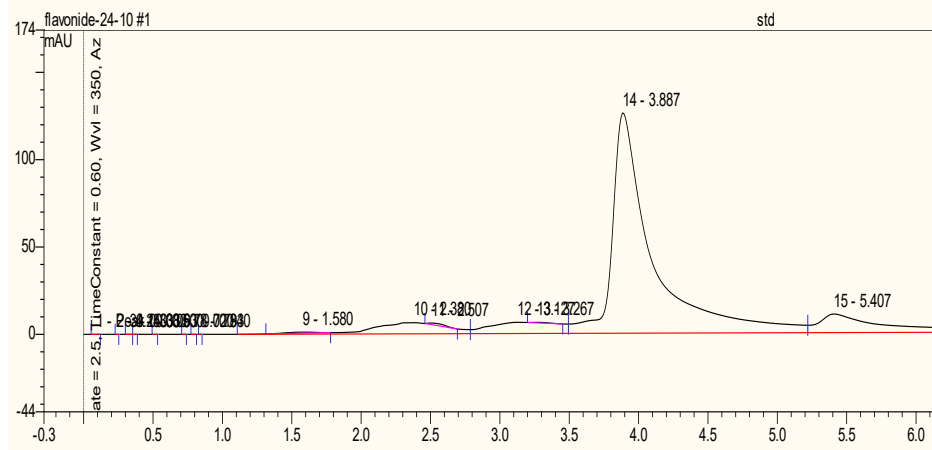


Figure 1: Standard Chromatogram of Morin.

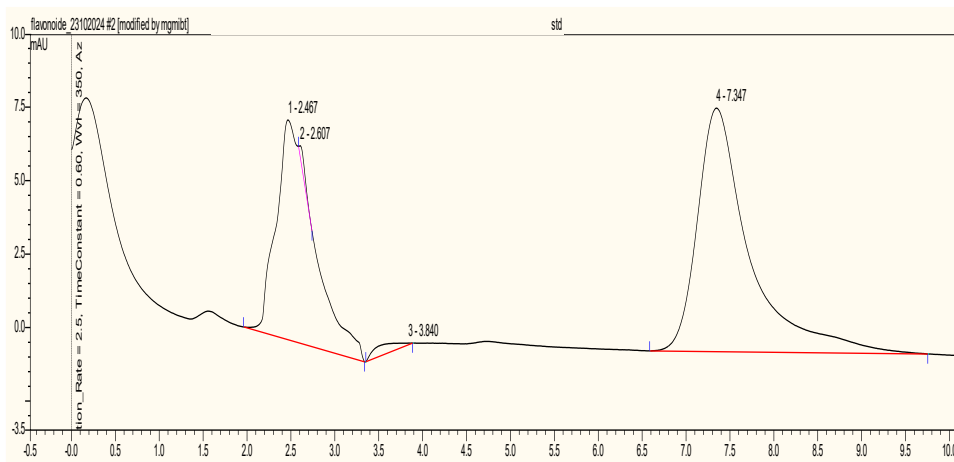


Figure 2: Standard Chromatogram of Naringin.

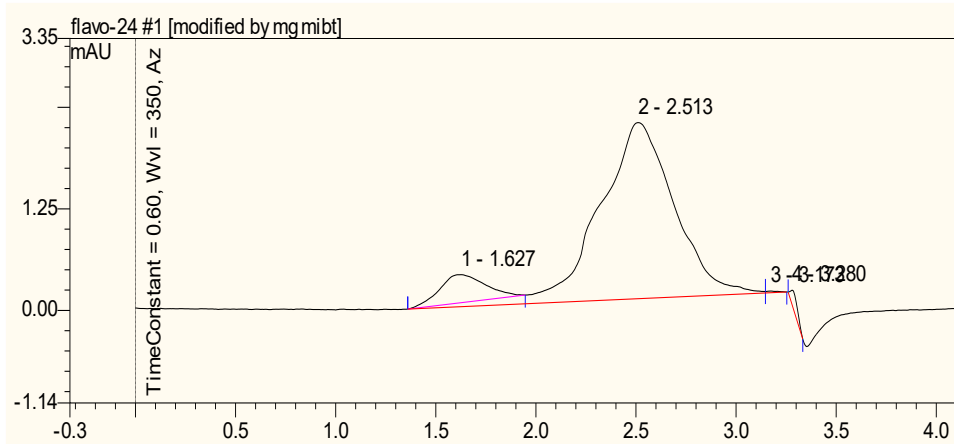


Figure 3: Standard Chromatogram of Quercetin.

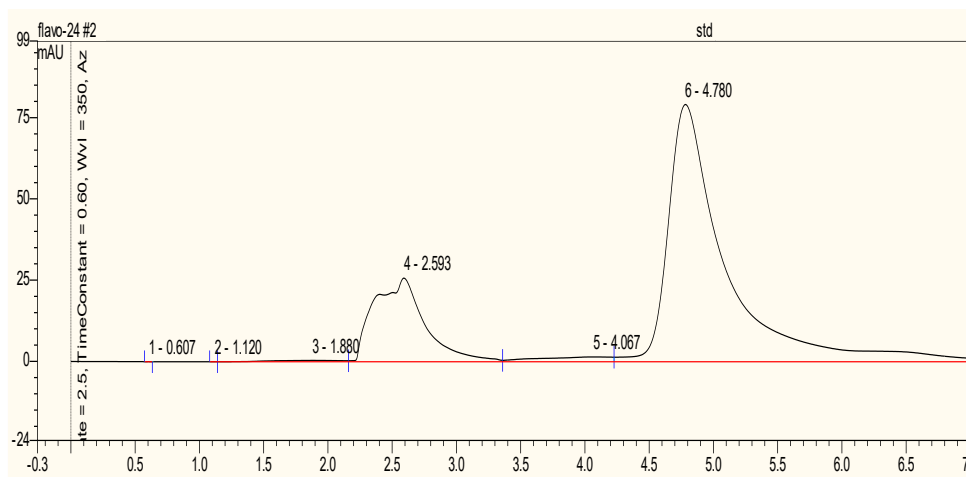


Figure 4: Standard Chromatogram of rutin.

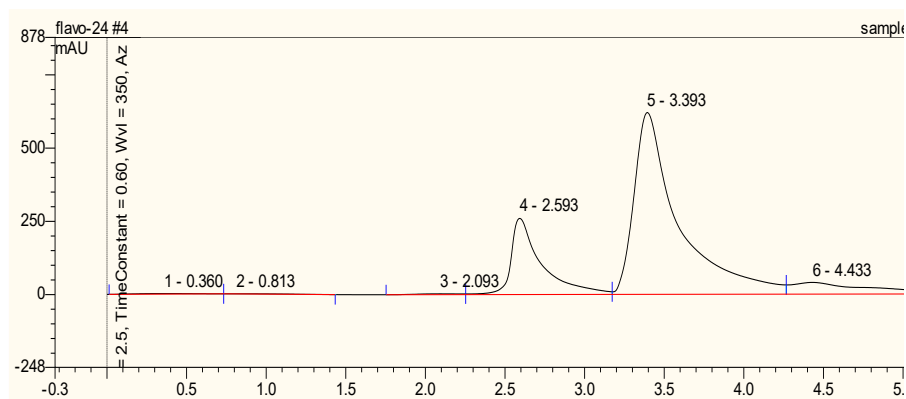


Figure 5: Chromatogram of Mango.

Blackberry leaf extract exhibited all four flavonoids, with retention times of morin at 3.640 min, naringin at 7.840 min, quercetin at 2.567 min, and rutin at 4.480 min (Figure 6). The peak areas were recorded as 1.7572, 189.5961, 48.1537, and 12.0042 mAUmin, respectively, suggesting that naringin was the most abundant flavonoid in blackberry leaves. Similarly, guava leaf extract followed the standard retention times, with naringin at 7.13 min and quercetin at 2.527 min, showing peak areas of 189.5961 mAUmin and 48.1537 mAUmin, respectively. These values indicate that naringin was more prominent than quercetin in guava leaves, consistent with previous reports on its high flavonoid content (Figure 8).

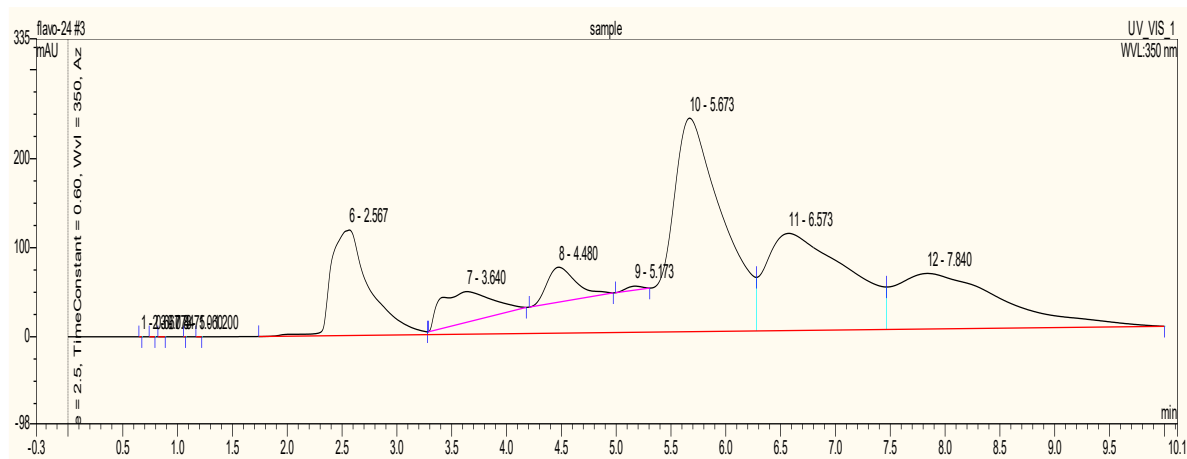


Figure 6: Chromatogram of blackberry.

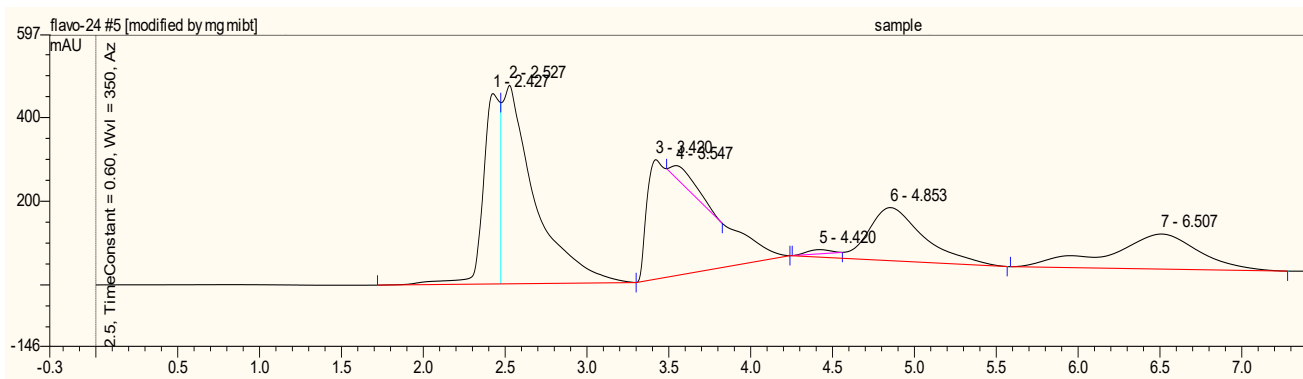


Figure 7: Chromatogram of *Pongamia pinnata* (L.) Pierre.

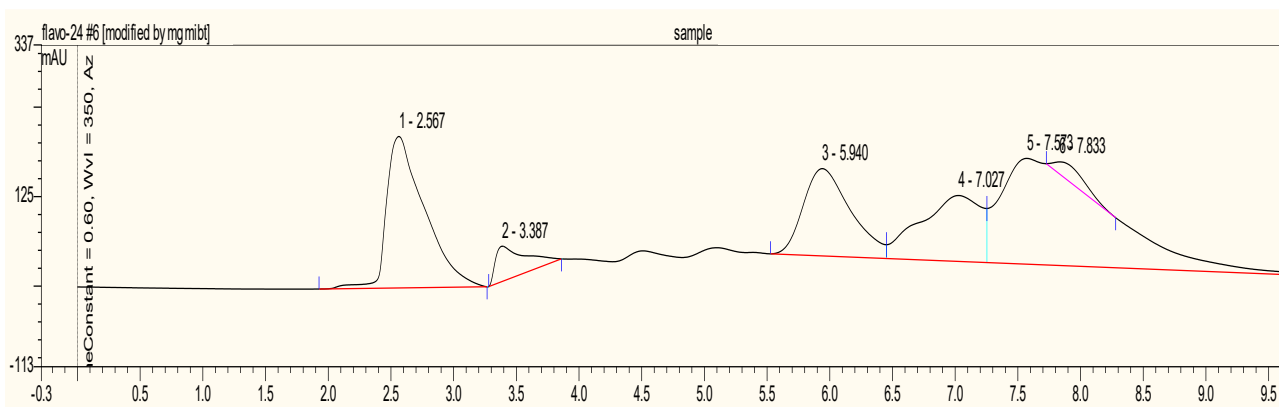


Figure 8: Chromatogram of *Psidium guajava* Linn.

For azadirachtin, the standard is analyzed using an RP column with a mobile phase of methanol and water (60:40), a detection wavelength of 214 nm, and a column temperature of 37°C. The retention time for the standard azadirachtin is recorded at 2.88 min, with a peak area of 33.7721 mAUmin (Figure 9). Comparatively, neem leaf samples show distinct variations in azadirachtin retention times and concentrations depending on their drying conditions. Dried neem leaves (Sample 1) exhibit a retention time of 3.327 min with a peak area of 197.78 mAUmin, corresponding to an azadirachtin concentration of 5.85 µg/mL (Figure 10). Semi-dried leaves (Sample 2) have a retention time of 3.253 min and a significantly higher peak area of 679.7476 mAUmin, indicating an azadirachtin concentration of 20.12 µg/mL (Figure 11). Fresh leaves (Sample 3) have a retention time of 2.305 min and a peak area of 395.5559 mAUmin, corresponding to a concentration of 11.71 µg/mL (Figure 12). These results suggest that semi-dried neem leaves retain the highest concentration of azadirachtin, while dried leaves exhibit the lowest concentration.

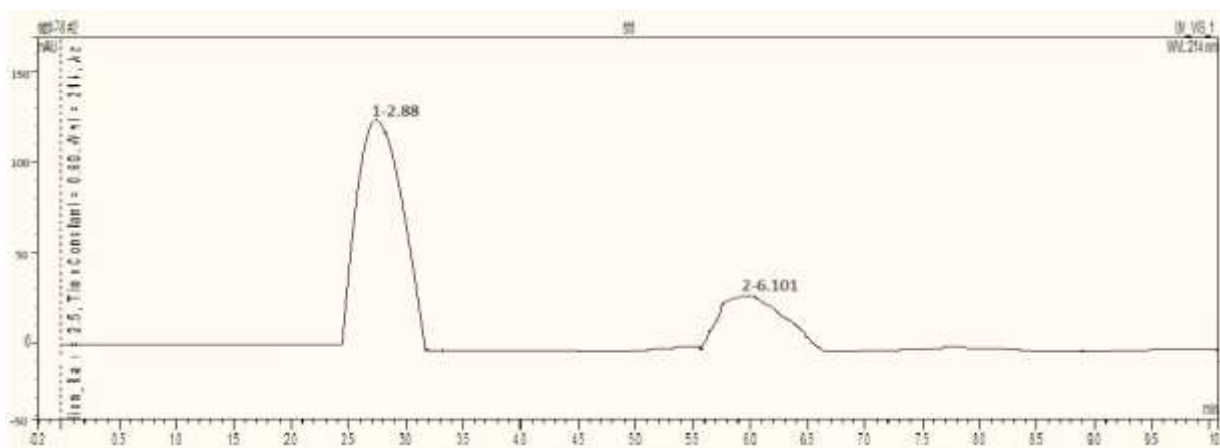


Figure 9: Chromatogram of Azadirachtin Standard

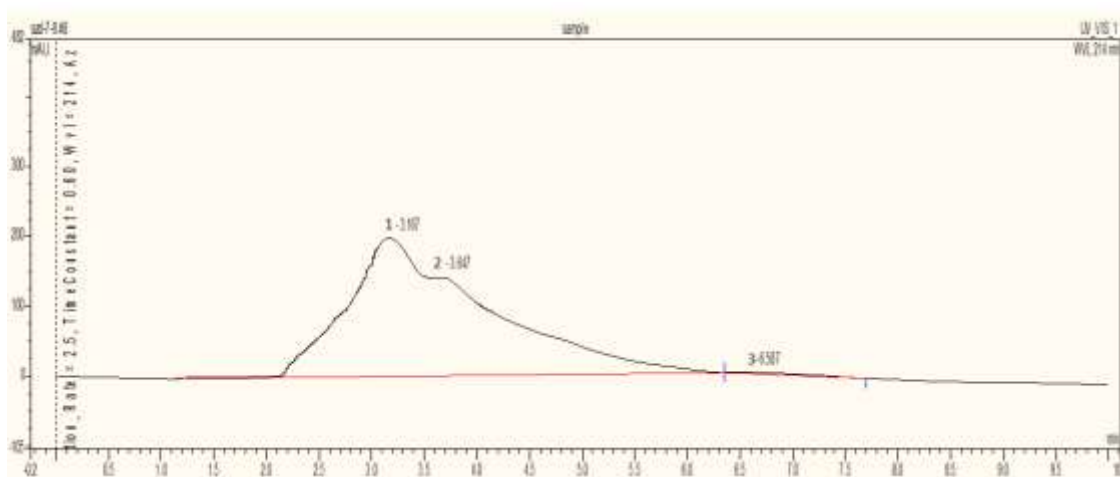


Figure 10: Chromatogram for Sample 1 (Fully dried neem leaves)

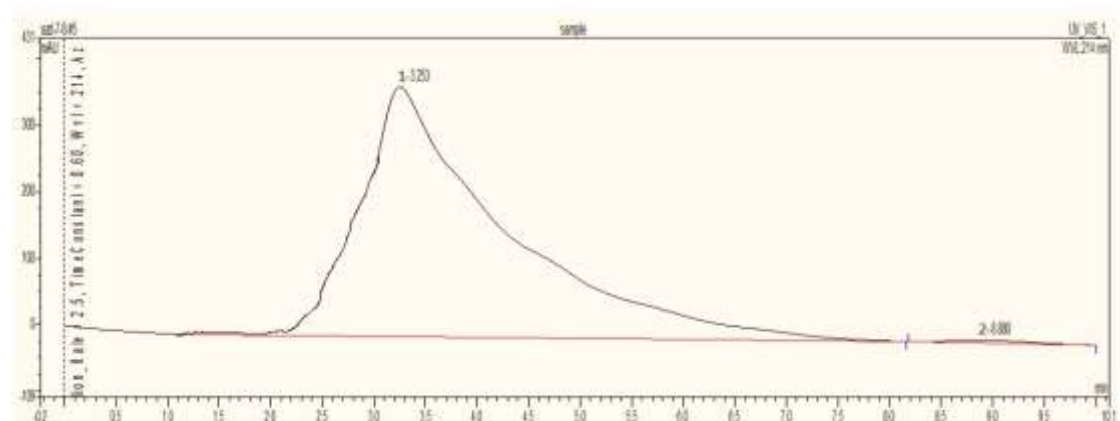


Figure 11: Chromatogram of Sample 2 (Semi-dried neem leaves)

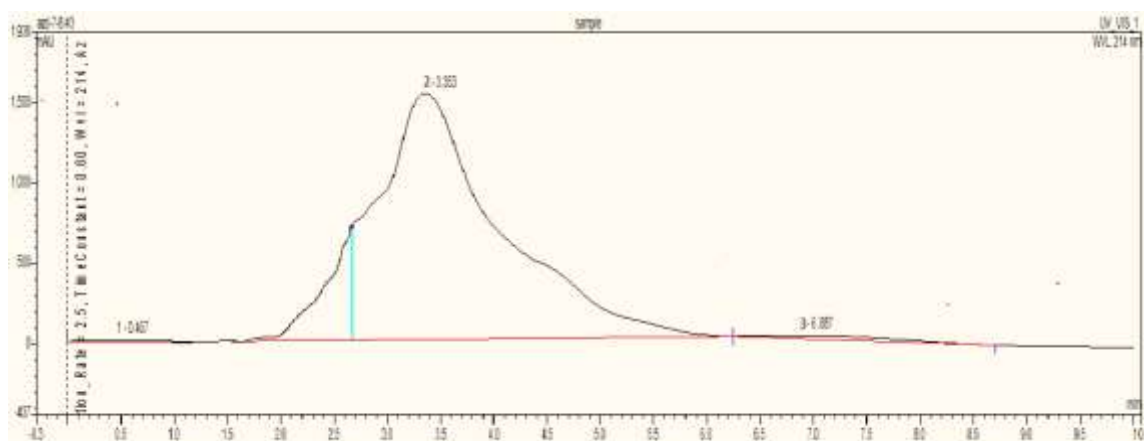


Figure 12: Chromatogram of Sample 3 (Fresh neem leaves)

DISCUSSION

The current study highlights the diverse distribution and quantification of flavonoids in *Mangifera indica* (Mango), *Psidium guajava* (Guava), *Rubus fruticosus* (Blackberry), *Pongamia pinnata* (Pongamia), and terpenoids in *Azadirachta indica* (Neem) plant leaves using High-Performance Liquid Chromatography (HPLC). Quercetin is the most prominent flavonoid present in all the plant leaves. Among the tested plants, *Pongamia pinnata* exhibited the highest diversity of flavonoids, containing morin, naringin, quercetin, and

rutin. Blackberry (*Rubus fruticosus*) and Mango (*Mangifera indica*) also showed notable concentrations of quercetin and morin [16]. Guava (*Psidium guajava*) leaves were particularly rich in naringin and quercetin [8].

The quantification of azadirachtin in *Azadirachta indica* (Neem) leaves revealed significant variation based on drying conditions, with semi-dried leaves exhibiting the highest concentration (20.12 µg/mL, RT = 3.253 min), followed by fresh leaves (11.71 µg/mL, RT = 2.305 min) and dried leaves (5.85 µg/mL, RT = 3.327 min) [15]. This suggests that drying methods influence the retention of bioactive compounds, a factor that should be considered in extraction and formulation processes [11]. The results also support existing literature, which identifies neem kernels as a more concentrated source of azadirachtin compared to leaves [10]. Nonetheless, the presence of this terpenoid in neem leaves highlights their continued relevance in eco-friendly pest control and medicinal applications [9]. The differences in flavonoid and terpenoid distribution among the studied plants underscore the influence of species-specific metabolic pathways and environmental factors on phytochemical accumulation [13]. These findings are consistent with previous studies on the phytochemical composition of these plants, which indicate their beneficial effects on human health, including anti-inflammatory, anticancer, and cardio protective properties [1].

CONCLUSION

These study successfully evaluated and quantified key flavonoids (morin, naringin, quercetin, and rutin) and the terpenoid azadirachtin in selected plant leaves using High-Performance Liquid Chromatography (HPLC). The findings reveal that quercetin is the most prominent flavonoid across the analyzed plant samples, while naringin and morin also show notable concentrations, particularly in guava, blackberry, and mango leaves. Among the tested species, *Pongamia pinnata* exhibited the highest diversity of flavonoids, making it a potential source for multiple bioactive compounds. Whereas the presence of azadirachtin in neem leaves varied significantly because of drying conditions, semi-dried leaves exhibit the highest concentration of Azadirachtin. The results contribute to the growth of knowledge on plant-based bioactive compounds and their applications in pharmaceutical, nutraceutical, and agricultural industries. Future studies should focus on optimizing extraction techniques to enhance compound yield, investigating potential synergistic effects among flavonoids and terpenoids, and exploring their bioavailability and therapeutic efficacy in clinical studies.

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REFERENCES

- [1] Antioxidant and antimutagenic potential of *Psidium guajava* leaf extract Maryam Zahin, Iqbal Ahmad & Farrukh Aqil to cite this article: Maryam Zahin, Iqbal Ahmad & Farrukh Aqil (2017) Antioxidant and antimutagenic potential of *Psidium guajava* leaf extracts, *Drug and Chemical Toxicology*, 40:2, 146-153, DOI: 10.1080/01480545.2016.1188397.
- [2] Alzohairy, M. A. (2020). Therapeutic roles of *Azadirachta indica* (Neem) and their active constituents in disease prevention and treatment. *Evidence-Based Complementary and Alternative Medicine*, 7382506. <https://doi.org/10.1155/2016/7382506>.
- [3] Aggarwal, M. (2018). *Experiment-27: Determination of Reichert Meissl (RM) value and Polenske value (PV) in oils and fats*. Indira Gandhi National Open University (IGNOU). <http://egyankosh.ac.in/handle/123456789/43431>
- [4] Anya, A. U., Chioma, N. N., & Obinna, O. (2012). Optimized reduction of free fatty acid content on neem seed oil, for biodiesel production. *Journal of Basic and Applied Chemistry*, 2(4), 21-28.
- [5] Ayoola, A. A., Efevbokhan, V. C., Bafuwa, O. T., & David, O. T. (n.d.). *A search for alternative solvent to hexane during neem oil extraction*. Chemical Engineering Department, Covenant University, Ota, Nigeria. <https://doi.org/10.24001/ijaems>.
- [6] Ambrosina, P., Fresa, R., Fogliano, V., Monti, S. M., & Ritieni, A. (1999). Extraction of azadirachtin A from neem seed kernels by supercritical fluid and its evaluation by HPLC and LC/MS. *Journal of Agricultural and Food Chemistry*, 47(12), 5252-5256. <https://doi.org/10.1021/jf9905368>.

- [7] *Blackberry | Description, Types, Nutrition, Uses, & Facts | Britannica*. (2024). Encyclopedia Britannica. <https://www.britannica.com/plant/blackberry-fruit>
- [8] Bader Ul Ain, H., Tufail, T., Javed, M., Tufail, T., Arshad, M. U., Hussain, M., Gull Khan, S., Bashir, S., Al Jbawi, E., & Abdulaali Saewan, S. (2022). Phytochemical profile and pro-healthy properties of berries. *International Journal of Food Properties*. <https://doi.org/10.1080/10942912.2022.2096062>
- [9] Begum, S., Hassan, S. I., Ali, S. N., & Siddiqui, B. S. (2004). Chemical constituents from the leaves of *Psidium guajava*. *Natural Product Research*, 18(2), 135-140.
- [10] Contributors to Wikimedia projects. (2024). *Blackberry - Wikipedia*. Wikimedia Foundation, Inc. <https://en.wikipedia.org/wiki/Blackberry>.
- [11] Dr. Liji Thomas, MD. (2023). *What makes blackberries a superfood?* News-Medical. <https://www.news-medical.net/news/20230406/What-makes-blackberries-a-superfood.aspx>
- [12] Daswani, P. G., Gholkar, M. S., & Birdi, T. J. (2017). *Psidium guajava*: A single plant for multiple health problems of rural Indian population. *Pharmacognosy reviews*, 11(22), 167.
- [13] Dubhashi, S., Pranay, V., Singaiah, M., Satwik, J., Prasad, V. V. L. N., & Diwan, P. V. (2013). Studies on extraction and HPLC analysis of azadirachtin from kernels of neem seeds. *Journal of Advanced Pharmacy Education & Research*, 3(1), 57-60.
- [14] Das, P., Sharma, N., Puzari, A., et al. (2021). Synthesis and characterization of neem (*Azadirachta indica*) seed oil-based alkyd resins for efficient anticorrosive coating application. *Polymer Bulletin*, 78, 457-479. <https://doi.org/10.1007/s00289-020-03120-8>
- [15] Dr. Raja Kumar Parabathina*, Sunil Kothargasti, Vishal Lolge, Nidhi Dubey, Sanika Girgaonkar, Studies On The Evaluation Of Flavonoids (Morin, Naringin, Quercetin & Rutin) In Pongamia Pinnata (L.) Pierre Leaves By HPLC, *Int. J. of Pharm. Sci.*, 2024, Vol 2, Issue 11, 878-883. <https://doi.org/10.5281/zenodo.14197141>
- [16] Dr. Raja Kumar Parabathina*, Sunil Kothargasti, Vishal Lolge, Nidhi Dubey, Sanika Girgaonkar, Studies on the Evaluation of Flavonoids (Morin, Naringin, Quercetin & Rutin) in Blackberry Leaves By HPLC, *Int. J. of Pharm. Sci.*, 2024, Vol 2, Issue 11, 904-909. <https://doi.org/10.5281/zenodo.14202760>
- [17] Dr. Raja Kumar Parabathina*, Nidhi Dubey, Sanika Girgaonkar, Vishal Lolge, Sunil Kothargasti, Evaluation And Determination of Azadirachtin and Fatty Acid Constants from The Leaves of *Azadirachta Indica*, *Int. J. of Pharm. Sci.*, 2024, Vol 2, Issue 12, 988-995. <https://doi.org/10.5281/zenodo.14328527>
- [18] Dr. Raja Kumar Parabathina*, Sunil Kothargasti, Vishal Lolge, Nidhi Dubey, Sanika Girgaonkar, Studies on the evaluation of flavonoids (Morin, Naringin, Quercetin & Rutin) in Mango leaves by HPLC, *Int. J. of Pharm. Sci.*, 2024, Vol 2, Issue 11, 884-889. <https://doi.org/10.5281/zenodo.14197490>.
- [19] Dr. Raja Kumar Parabathina*, Sunil Kothargasti, Vishal Lolge, Nidhi Dubey, Sanika Girgaonkar, Studies on the Evaluation of Flavonoids (Morin, Naringin, Quercetin & Rutin) in *Psidium Guajava* Linn. Leaves By HPLC, *Int. J. of Pharm. Sci.*, 2024, Vol 2, Issue 11, 849-855. <https://doi.org/10.5281/zenodo.14185327>.
- [20] Esparza-Diaz, G., Villaneva- Jimenez., Lopez Collado, J., & Rodriguez- Lagunes, D. (2010). Azadirachtin extraction using cold press and Soxhlet methods. *Biopesticides International*, 6(1), 45-51.
- [21] euphoric admin. (2022). *Top 5 Benefits of Blackberry Leaf*. Euphoric Herbals. <https://www.euphoricherbals.com/blogs/blog/benefits-of-blackberry-leaf>
- [22] Fernandes, S., Santos, N., & Morgado, J. (2020). Chemistry, bioactivities, extraction, and analysis of azadirachtin: State-of-the-art. *Fitoterapia*, 134, 104732. <https://doi.org/10.1016/j.fitote.2019.02.006>
- [23] Forim, M. R., da Silva, M. F. G. F., Cass, Q. B., Fernandes, J. B., & Vieira, P. C. (2010). Simultaneous quantification of azadirachtin and 3-tigloylazadirachtol in Brazilian seeds and oil of *Azadirachta indica*: Application to quality control and marketing. *Analyst*, 135(9), 2334-2340. <https://doi.org/10.1039/c0ay00008f>
- [24] Gil-Martínez, L., Mut-Salud, N., Ruiz-García, J. A., Falcón-Piñero, A., Maijó-Ferré, M., Baños, A., De la Torre-Ramírez, J. M., Guillamón, E., Verardo, V., & Gómez-Caravaca, A. M. (2023). Phytochemicals Determination, and Antioxidant, Antimicrobial, Anti-Inflammatory and Anticancer Activities of Blackberry Fruits. *Foods*. <https://doi.org/10.3390/foods12071505>
- [25] Gutiérrez, R. M. P., Mitchell, S., & Solis, R. V. (2008). *Psidium guajava*: A review of its traditional uses, phytochemistry and pharmacology. *Journal of ethnopharmacology*, 117(1), 1-27.

- [26] Giuseppe Coglienti. (2024). *The chemistry of Mango - Blog — Scentspiracy*. scentspiracy. <https://www.scentspiracy.com/blog/the-chemistry-of-mango>.
- [27] Heppy, F., Mulyana, R., Syah, N. A., & Tjandrawinata, R. R. (2023). The effect of Psidium guajava Leaves' extract for mild and asymptomatic coronavirus Disease-19. *Saudi Pharmaceutical Journal*, 31(4), 592-596.
- [28] Hundie, K., Abdissa, D., & Bayu, A. (2022). Extraction, optimization, and characterization of neem seed oil via Box-Behnken design approach. *Journal of the Turkish Chemical Society Section A: Chemistry*, 9(2), 513-526. <https://doi.org/10.18596/jotcsa.1039997>
- [29] Hamadou, B., Djomdi, F., Falama, R. Z., Cedric, D., Guillaume, P., Pascal, D., & Philippe, M. (2020). Influence of physicochemical characteristics of neem seeds (*Azadirachta indica* A. Juss) on biodiesel production. *Biomolecules*, 10(4), 616. <https://doi.org/10.3390%2Fbiom10040616>
- [30] Hiremath, S. I., Menasinakai, A. S., Gharge, S., Koganole, P., & Mali, N. (2023). Quality control and standardization of azadirachtin in herbal medicines by spectroscopic and chromatographic techniques. *International Journal of Creative Research Thoughts (IJCRT)*, 11(2), 210-220. <https://doi.org/10.13140/RG.2.2.31278.27207​>
- [31] Huang, H. P., & Morgan, E. D. (1990). Analysis of azadirachtin by supercritical fluid chromatography. *Journal of Chromatography A*, 519, 137-143. [https://doi.org/10.1016/S0021-9673\(01\)84011-4](https://doi.org/10.1016/S0021-9673(01)84011-4)
- [32] Islas, J. F., Acosta, E., G-Buentello, Z., Delgado-Gallegos, J. L., Moreno-Treviño, M. G., Escalante, B., & Moreno-Cuevas, J. E. (2020). An overview of neem (*Azadirachta indica*) and its potential impact on health. *Journal of Functional Foods*, 74, 104171. <https://doi.org/10.1016/j.jff.2020.104171>
- [33] June 7, 2024. (2024). *Journey Foods 2024*. journeyfoods.io. <https://www.journeyfoods.io/blog/blackberry-leaf-a-berry-underlooked-food-product>.
- [34] Joshua Kaiser. (2024). *Blackberry & Fermented Blackberry Leaves*. Rishi Tea & Botanicals. <https://rishi-tea.com/blogs/journal/blackberry-fermented-blackberry-leaves>.
- [35] Jamieson, S., Wallace, C. E., Das, N., Bhattacharyya, P., & Bishayee, A. (2022). Guava (*Psidium guajava* L.): a glorious plant with cancer-preventive and therapeutic potential. *Critical reviews in food science and nutrition*, 63(2), 192-223.
- [36] Kumar, G. T. J., Andrews, B. S. A., & Abbaraju, V. D. N. K. (2023). *The significance of system suitability in high-performance liquid chromatography (HPLC) analysis: Ensuring accurate and reliable results*. *International Journal for Research in Applied Science & Engineering Technology*, 11(11). <https://doi.org/10.22214/ijraset.2023.56435>.
- [37] Kumar, M., Saurabh, V., Tomar, M., Hasan, M., Changan, S., Sasi, M., Maheshwari, C., Prajapati, U., Singh, S., Prajapat, R. K., Dhumal, S., Punia, S., Amarowicz, R., & Mekhemar, M. (2021). Mango (*Mangifera indica* L.) Leaves: Nutritional Composition, Phytochemical Profile, and Health-Promoting Bioactivities. *Antioxidants*. <https://doi.org/10.3390/antiox10020299>.
- [38] Kilani-Morakchi, S., Morakchi-Goudjil, H., & Sifi, K. (2021). Azadirachtin-based insecticide: Overview, risk assessments, and future directions. *Frontiers in Agronomy*, 3, 676208. <https://doi.org/10.3389/fagro.2021.676208>
- [39] Lauricella, M., Emanuele, S., Calvaruso, G., Giuliano, M., & D'Anneo, A. (2017). Multifaceted Health Benefits of *Mangifera indica* L. (Mango): The Inestimable Value of Orchards Recently Planted in Sicilian Rural Areas. *Nutrients*. <https://doi.org/10.3390/nu9050525>.
- [40] Lucinda Honeycutt. (2019). *What Are the Benefits of Blackberry Leaves? | Livestrong.com*. Livestrong.com. <https://www.livestrong.com/article/257328-what-are-the-benefits-of-blackberry-leaves/>
- [41] Liauw, M. Y., Natan, F. A., Widiyanti, P., Ikasari, D., Indraswati, N., & Soetaredjo, F. E. (2008). Extraction of neem oil (*Azadirachta indica* A. Juss) using n-hexane and ethanol: Studies of oil quality, kinetic and thermodynamic. *ARPN Journal of Engineering and Applied Sciences*, 3(3), 49-54.
- [42] Maldonado-Celis, M. E., Yahia, E. M., Bedoya, R., Landázuri, P., Loango, N., Aguillón, J., Restrepo, B., & Guerrero Ospina, J. C. (2019). Chemical Composition of Mango (*Mangifera indica* L.) Fruit: Nutritional and Phytochemical Compounds. *Frontiers in Plant Science*. <https://doi.org/10.3389/fpls.2019.01073>
- [43] Metwally, A. M., Omar, A. A., Harraz, F. M., & El Sohafy, S. M. (2010). Phytochemical investigation and antimicrobial activity of *Psidium guajava* L. leaves. *Pharmacognosy magazine*, 6(23), 212.
- [44] Nnam, R. E., Okoro, O. I., Oko, A. N., & Oji, C. O. (2021). Crop protectant from pest by neem (*Azadirachta indica*) oil bioinsecticide. *Central Asian Journal of Theoretical and Applied Sciences*, 2(2), 63-76. ISSN 2660-5317.
- [45] Paczkowska-Walendowska, M., Gościński, A., Szymanowska, D., Sz wajgier, D., Baranowska-Wójcik, E., Szulc, P., Dreczka, D., Simon, M., & Cielecka-Piontek, J. (2021). Blackberry Leaves as New

- Functional Food? Screening Antioxidant, Anti-Inflammatory and Microbiological Activities in Correlation with Phytochemical Analysis. *Antioxidants*. <https://doi.org/10.3390/antiox10121945>
- [46] Rajakumar, P., Muralinath, E., Lakshmana S.P., Harikrishna, V.V.S.N., Shanthi, S.K., (2011) Effects of the vitamin-e, morin, quercetin against doxorubicin in rabbit: a hematological study. *Research journal of pharmaceutical biological and chemical sciences* 2(3), 74.
- [47] Rajakumar, P., Muralinath, E., Kishore, G., and Kaza, S.R. (2011) Effect of Vitamin- E, morin, quercetin, and rutin against dox induced oxidative stress. *International Journal of Applied Biology and Pharmaceutical Technology* 2(1), 399.
- [48] Rajakumar, P., Kishore, G., and Srinivasa, G.R., (2011). Studies on changes in the hematology of rabbits treated with vitamin- e, morin, rutin, and quercetin, against doxorubicin. *International journal of biotechnology and bioengineering research*, 2(2), 307-317.
- [49] Sivankalyani, V., Feygenberg, O., Diskin, S., Wright, B., & Alkan, N. (2016). Increased anthocyanin and flavonoids in mango fruit peel are associated with cold and pathogen resistance. *Postharvest Biology and Technology*. <https://doi.org/10.1016/j.postharvbio.2015.08.001>.
- [50] Sampath Kumar, N. S., Sarbon, N. M., Rana, S. S., Chintagunta, A. D., Prathibha, S., Ingilala, S. K., ... & Dirisala, V. R. (2021). Extraction of bioactive compounds from Psidium guajava leaves and its utilization in preparation of jellies. *AMB Express*, 11, 1-9.
- [51] Subramanian, S., Salleh, A., & Bachmann, R. (2020). Simultaneous extraction and separation of oil and azadirachtin from seeds and leaves of *Azadirachta indica* using binary solvent extraction. *Natural Product Sciences*, 25(2), 150-158. <https://doi.org/10.20307/nps.2019.25.2.150>
- [52] Sarkar, S., Singh, R. P., & Bhattacharya, G. (2021). Exploring the role of *Azadirachta indica* (neem) and its active compounds in the regulation of biological pathways: An update on molecular approach. *3 Biotech*, 11(4), 178. <https://doi.org/10.1007/s13205-021-02745>
- [53] Sandanasamy, J., Nour, A. H., Tajuddin, S. N., & Nour, A. H. (2013). Fatty acid composition and antibacterial activity of neem (*Azadirachta indica*) seed oil. *The Open Conference Proceedings Journal*, 4, 43-48.
- [54] Shikha Pandhi, Arvind Kumar and Dinesh Chandra Rai (2022). Efficacy evaluation of extraction technologies for guava (*Psidium guajava* L.) leaves extract. *Ann. Phytomed.*, 11(1):413-418. <http://dx.doi.org/10.54085/ap.2022.11.1.47>.
- [55] Sen, A.K., Sen, D.B., Maheshwari, R.A. (2020). Extraction, Isolation, and Quantitative Determination of Flavonoids by HPLC. In: Sen, S., Chakraborty, R. (eds) *Herbal Medicine in India*. Springer, Singapore. https://doi.org/10.1007/978-981-13-7248-3_21.
- [56] TFNet News Compilation. (2016). *MANGO – Name, Taxonomy, Botany – TFNet – International Tropical Fruits Network*. itfnet.org. <https://www.itfnet.org/v1/2016/05/mango-name-taxonomy-botany-2>
- [57] Tesfaye, B., & Tefera, T. (2017). Extraction of essential oil from neem seed by using the Soxhlet extraction methods. *International Journal of Advanced Engineering, Management and Science (IJAEMS)*, 3(6), 646. <https://doi.org/10.24001/ijaems.3.6.5>
- [58] Tesfaye, B., Tefera, T., Misikir, O., & Tsegaye, G. (2018). Extraction and comparison of essential oil from neem seed by using Soxhlet extraction and simple distillation methods. *International Journal of Engineering Technologies and Management Research*, 5(9), 74–81. <https://doi.org/10.29121/ijetmr.v5.i9.2018.291>
- [59] Varzaru, I., Oancea, A. G., Vlaicu, P. A., Saracila, M., & Untea, A. E. (2023). Exploring the Antioxidant Potential of Blackberry and Raspberry Leaves: Phytochemical Analysis, Scavenging Activity, and In Vitro Polyphenol Bioaccessibility. *Antioxidants*. <https://doi.org/10.3390/antiox12122125>
- [60] Wu, Y., Huang, X., Yang, H., Zhang, S., Lyu, L., Li, W., & Wu, W. (2023). Analysis of flavonoid-related metabolites in different tissues and fruit developmental stages of blackberry based on metabolome analysis. *Food Research International*. <https://doi.org/10.1016/j.foodres.2022.112313>.
- [61] Weli, A., Al-Kaabi, A., Al-Sabahi, J., Said, S., Hossain, M. A., & Al-Riyami, S. (2019). Chemical composition and biological activities of the essential oils of *Psidium guajava* leaf. *Journal of King Saud University-Science*, 31(4), 993-998.
- [62] Yahaya, S., Sani, A., Murtala, Y., Momoh, H., & Mohammed, S. (2022). Determination of fatty acids and physicochemical properties of neem (*Azadirachta indica* L.) seed oil extracts. *Dutse Journal of Pure and Applied Sciences*, 8(2), 149-160. <https://dx.doi.org/10.4314/dujopas.v8i1a.16>.