

Phytochemical Screening and Bioactive Compound Identification in Mango (*Mangifera indica* L.) Seed Extracts

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Abstract- *The mango (*Mangifera indica* L.) seeds are becoming a promising source of phytochemicals with important biological activities. Phytochemical screening and bioactive compounds identification in mango seed extracts were done in this study. Seed kernels were collected, dried, ground and extracts were prepared with organic solvents and analysed for major secondary metabolites. The screening showed the presence of Alkaloids, Saponins, Glycosides, Carbohydrates and sugars, Tannins, Flavonoids. Steroids, Amino Acid, Protein and Phenolic compounds. High levels of phenolic and flavonoid contents proved high antioxidant potential and can be utilised in pharmaceutical, nutraceutical and functional food industries. In this context, mango seeds are considered as a cheap and sustainable source of valuable bioactive compounds that are often discarded as waste. Further studies on purification and biological evaluation are required to produce new therapeutic agents and products.*

Keywords: *Mango Seed, Phytochemical Screening, Bioactive Compounds, Phenolic Compounds, Flavonoids, Antioxidant Activity, Agro-Industrial Waste.*

I. INTRODUCTION

Medicinal plants are an important source of bioactive compounds which have received much attention because of their therapeutic, nutraceutical and pharmaceutical uses. These natural products contain a wide range of phytochemicals such as polyphenols, flavonoids, tannins, alkaloids, terpenoids, xanthenes and carotenoids with antioxidant, antimicrobial, anti-inflammatory, antidiabetic and anticancer activities (Masibo and He, 2009).

Mangifera indica L. (mango), a member of Anacardiaceae family, is one of the most economically important tropical fruit crops. India is the world's largest producer of mango and generates

large amounts of agro-industrial waste, mainly in the form of peels and seeds. Usually discarded during fruit processing, the kernels of mango seed are a potent source of bioactive phytochemicals with immense medicinal potential (Jahurul et al., 2015).

The mango seed contains a variety of phytochemicals including mangiferin (the main bioactive xanthone), gallic acid, ellagic acid, catechins, quercetin, kaempferol, tannins, phenolic acids, carotenoids and ascorbic acid. These compounds have strong antioxidant properties and are involved in several pharmacological activities (Masibo and He, 2008).

Phytochemical screening is the first step for characterisation of nature of secondary metabolites present in the plant extracts. These bioactive compounds were isolated and their structure was characterised by several advanced analytical techniques including Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC), Gas Chromatography–Mass Spectrometry (GC–MS), Liquid Chromatography–Mass Spectrometry (LC–MS), Fourier Transform Infrared Spectroscopy (FTIR), and Nuclear Magnetic Resonance (NMR) (Quintana et al., 2021). Ribeiro et al. (2016) reviewed the functional and nutritional properties of mango seed and showed that the kernels of mango seed have high antioxidant capacity, are rich in proteins, unsaturated fatty acids, polyphenols, and essential amino acids and are promising functional food ingredients. Quintana et al. (2021) discussed the recent extraction technologies and phytochemical characterisation methods of mango bioactive compounds. The review pointed out the importance of the extraction methods on the yield and the composition of phenolics, flavonoids, carotenoids and terpenoids. A systematic review of

phytochemicals present in different parts of mango by Yadav et al. (2022) reported that extracts from mango seed are rich in flavonoids, tannins, alkaloids, saponins, terpenoids, steroids and phenolic compounds which are responsible for antioxidant, antimicrobial, antidiabetic, anticancer, anti-inflammatory and hepatoprotective activities. Mango agro-industrial waste: Extraction, identification and quantification of bioactive compounds, recent advances Siller-Cepeda, and colleagues (2023). They revealed that mango seeds are rich sources of polyphenols and carotenoids and have significant antioxidant and antimicrobial properties with potential applications in functional foods and pharmaceuticals.

II. MATERIALS AND METHODS

Fresh mango (*Mangifera indica* L.) fruits were obtained from local orchards. Seeds were separated, thoroughly washed with distilled water, shade dried and ground to fine powder for further analysis (Harborne 1998).

Preparation of seed extract

Powdered seed kernel was extracted with solvents of increasing polarity (Ethanol Extract, Methanol, distilled water) by Soxhlet extraction or cold maceration. The extracts were filtered, concentrated under reduced pressure using a rotary evaporator and stored at 4°C (Soxhlet, 1879).

Preliminary Phytochemical Analysis

Qualitative phytochemical screening Alkaloids, Saponins, Glycosides, Carbohydrates and sugars, Tannins, Flavonoids. Steroids, Amino Acid, Protein and Phenolic compounds. was performed using standard protocols as described by Trease and Evans (2009).

Identification of Active Compounds

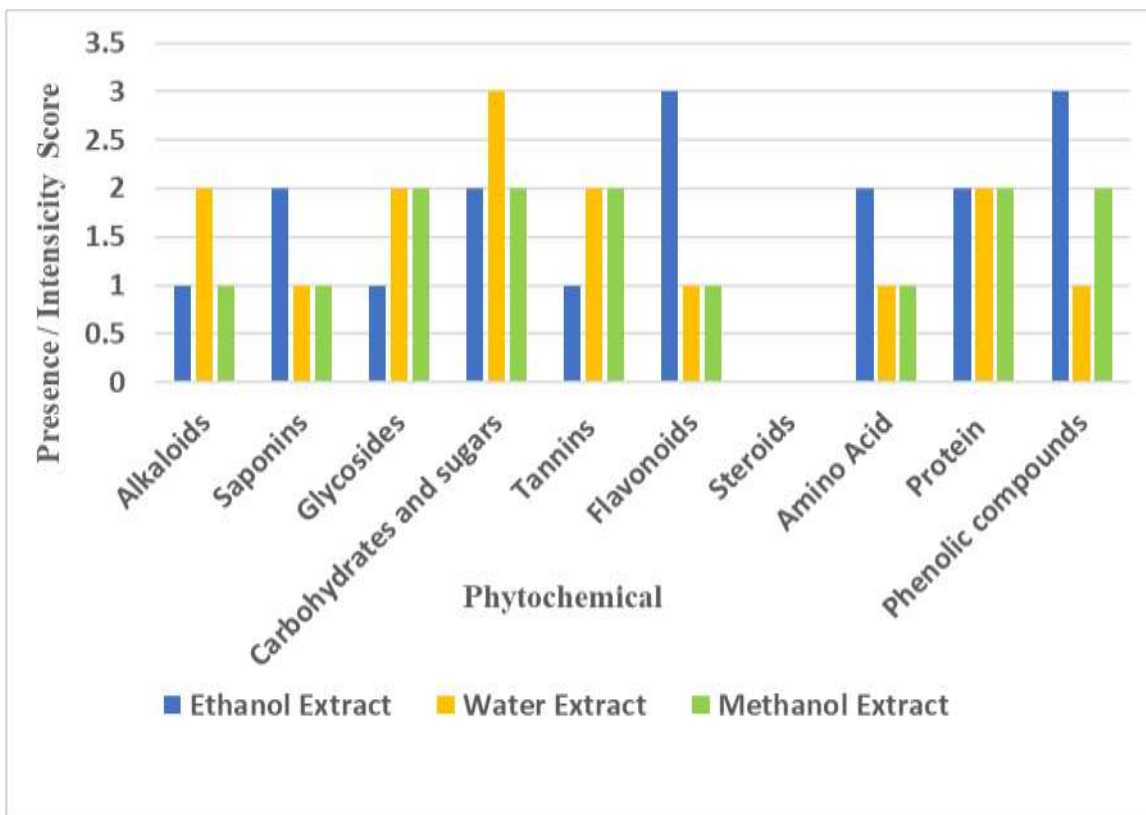
The purified compounds were characterised by chromatographic and spectroscopic techniques such as High-Performance Liquid Chromatography (HPLC) and Gas Chromatography – Mass Spectrometry (GC – MS) where applicable. Identification of compounds was done by comparing spectral data with published literature and standard databases (Skoog et al., 2018).

III. RESULT AND DISCUSSION

Table.2: Phytochemical screening of seeds & seed kernels extracts of powdered of Mango (*Mangifera indica* L.)

Test Name	Results of extracts of powdered seed in different soluble		
	Ethanol Extract	Water Extract	Methanol Extract
Alkaloids	1	2	1
Saponins	2	1	1
Glycosides	1	2	2
Carbohydrates and sugars	2	3	2
Tannins	1	2	2
Flavonoids	3	1	1
Steroids	0	0	0
Amino Acid	2	1	1
Protein	2	2	2
Phenolic compounds	3	1	2

0= Absent, 1= Present, 2= Strongly Present & 3 = Very Strongly Present



Graph No. 2 Phytochemical screening of seeds & seed kernels extracts of powdered of Mango kernel/stone is a good source of phenolic compounds, including the microelements like Se, Cu, and Zn. The anti-oxidant property of the mango kernel is due to its high content of polyphenols, sesquiterpenoids, phytosterols, and the microelements – Se, Cu, and Zn. Antioxidants present in mango kernel would be a potential source of natural antioxidants and can substitute the usage of chemically synthesized antioxidants in the food industry (Mwaurah et. Al., 2020). The various polyphenolic compounds in the mango kernel are tannin, gallic acid, coumarin, caffeic acid, vanillin, mangiferin, ferulic acid, cinnamic acid, and several unknown compounds. The extract of the mango kernel was found to contain high level of phenolics with 85.7% as methyl gallate (Maisuthisakul et al., 2014). Treating (soaking and heating) the mango kernel was found to reduce the mineral (Fe, Ca, P) and phytochemical (ascorbic acid, polyphenol, flavonoid, tannin) contents significantly (Ojha et al., 2019).

HPLC analysis of the seed extract of *Mangifera indica* revealed the presence of gallic acid, a major phenolic compound known for its antioxidant activity. A distinct chromatographic peak corresponding to gallic acid was observed at a retention time (RT) of 3.041 min with a peak area of 3,761,552.

"The HPLC chromatogram of *Mangifera indica* seeds & seed kernels extract exhibited a well-resolved peak corresponding to gallic acid at a retention time of 3.041 min. The peak area was recorded as 3,761,552, indicating the presence of a considerable amount of gallic acid in the extract. Gallic acid is one of the principal phenolic constituents reported in mango seed kernels and contributes significantly to their antioxidant, antimicrobial, and anti-inflammatory properties. Identification of gallic acid was achieved by comparing the retention time of the sample peak with that of a reference standard analyzed under identical chromatographic conditions."



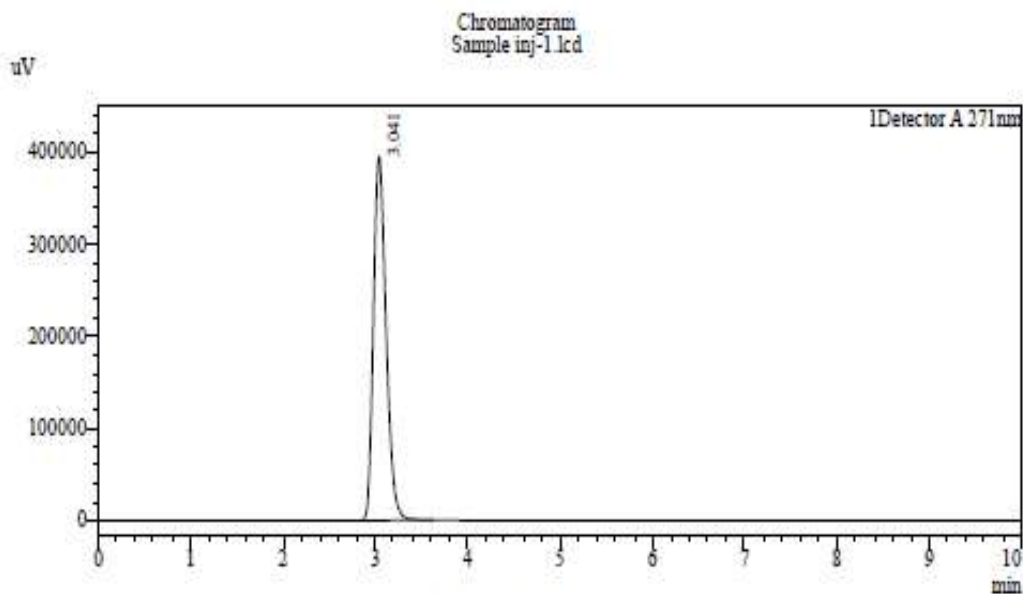
HPLC CHROMATOGRAM

Sample Information Sample inj-1.lcd

Sample Name : Gallic Acid
Sample ID : Sample inj-1
Data Filename : Sample inj-1.lcd
Method Filename : Gallic Acid_PM.lcm
Batch Filename : Gallic Acid_12032026.lcb
Column Detail : (250 mm X 4.6 mm) 5µm

Tray# : 01
Injection Volume : 20 µL
Date Acquired : 12/03/2026 13:23:14
Date Processed : 12/03/2026 17:39:26
Wave Length : 230 nm
Instrument ID : ANU-HP-22-02

Vial# : 03
Sample Type : Unknown
Acquired by : Teena Malviya
Processed By : Teena Malviya
Run Time : 10.0 min
Report File Name : DEFAULT1.r



Peak Table Sample inj-1.lcd

Peak#	Name	Ret. Time	Area	Area%
1	Gallic Acid	3.041	3761552	100.000
Total			3761552	100.000

Graph 10. HPLC result of *Mangifera indica* L. seeds & seed kernels extract

Table 12. HPLC result of *Mangifera indica* L. seeds & seed kernels extract

Compound	(min)	Area
Gallic Acid	3.041	3,761,552

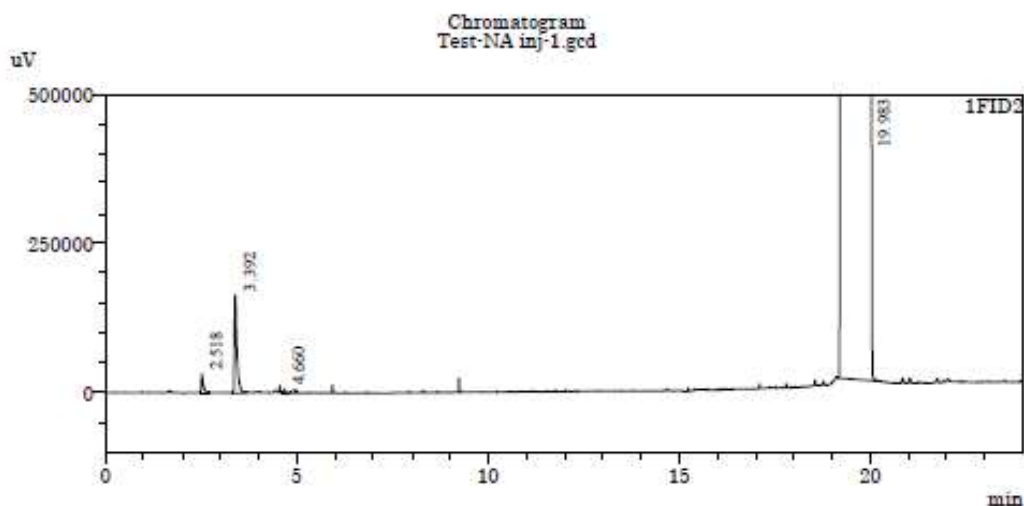
Identified	Retention Time	Peak
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GC CHROMATOGRAM

Sample Name : Gallic Acid
Sample ID : Test-NA inj-1
Data Filename : Test-NA inj-1.gcd
Method Filename : Gallic Acid_Residual Solvent.gcm
Batch Filename : Gallic Acid_Residual Solvent_12032026.gcb
Vial# : 05
Injection Volume : 1 uL
Date Acquired : 12/03/2026 15:09:51
Date Processed : 12/03/2026 16:11:41

Sample Information Test-NA inj-1.gcd
Acquired by : durgaprasad lavanshi
Processed By : durgaprasad lavanshi



Peak Table Test-NA inj-1.gcd

Peak#	Name	Ret. Time	Area	Area%
1	Methanol	2.518	42266	0.003
2	Ethanol	3.392	226438	0.015
3	Dichloromethane	4.660	12065	0.001
4	Dimethyl Sulphoxide	19.983	1531776870	99.982
Total			1532057639	100.000

Graph 12. GC result of Annonacin Isolated from *Mangifera indica* L. seeds & seed kernels Extract

Table 14. GC result of Annonacin Isolated from *Mangifera indica* L. seeds & seed kernels Extract

Peak No.	Residual Solvent	Retention Time (min)	Area	Area (%)
1	Methanol	2.518	42,266	0.003
2	Ethanol	3.392	226,438	0.015
3	Dichloromethane	4.660	12,065	0.001
4	Dimethyl Sulphoxide (DMSO)	19.983	1,531,776,870	99.982
Total	—	—	1,532,057,639	100.000

Gas Chromatography (GC) analysis was performed to determine the residual solvent content in the gallic acid-rich fraction isolated from *Mangifera indica* seed extract. The chromatogram revealed the presence of

methanol, ethanol, dichloromethane, and dimethyl sulphoxide (DMSO).

GC residual solvent analysis of the gallic acid-rich fraction isolated from *Mangifera indica* L. seed extract revealed four solvent peaks. Methanol, ethanol, and dichloromethane were detected at retention times of 2.518, 3.392, and 4.660 min, respectively, contributing only trace amounts to the total chromatographic area. Dimethyl sulphoxide (DMSO) was observed as the major peak at a retention time of 19.983 min with an area of 1,531,776,870, accounting for 99.982% of the total peak area.

"The very low area percentages of methanol (0.003%), ethanol (0.015%), and dichloromethane (0.001%) indicate efficient removal of extraction solvents during purification of the gallic acid fraction. The predominance of DMSO suggests that it served as the final solvent for dissolution of the isolated fraction prior to chromatographic analysis."

IV. CONCLUSION

The findings confirm that both seeds are valuable sources of bioactive secondary metabolites. *A. squamosa* seeds exhibited broader phytochemical richness, supporting earlier ethnomedicinal reports. *M. indica* seed kernels showed high antioxidant potential due to abundant polyphenols, consistent with previous literature highlighting their nutraceutical and food-industry relevance. Overall, the study supports the therapeutic and industrial potential of these plant seeds as natural sources of bioactive compounds.

"The presence of gallic acid in *M. indica* seeds & seed kernels extract agrees with previous phytochemical investigations, which reported high levels of phenolic compounds, especially gallic acid and gallotannins, in mango seed kernels. These compounds are considered responsible for the strong free-radical scavenging activity of the extract."

Overall, the phytochemical screening demonstrated that the powdered seeds & seed kernels of *Annona squamosa* contain a wide range of bioactive compounds, including alkaloids, saponins, tannins, flavonoids, steroids, amino acids, proteins, and phenolic compounds. Among the solvents tested,

water and methanol extracts exhibited higher extraction efficiency for several phytochemical groups, while ethanol extract was particularly effective in extracting steroids. The presence of these bioactive constituents supports the traditional medicinal use of *A. squamosa* seeds and suggests their potential as a source of natural therapeutic agents.

GC residual solvent analysis of the gallic acid-rich fraction isolated from *Mangifera indica* L. seed extract showed trace amounts of methanol (0.003%), ethanol (0.015%), and dichloromethane (0.001%), while dimethyl sulphoxide (DMSO) accounted for 99.982% of the total chromatographic area. These findings indicate effective removal of extraction solvents and good purity of the isolated gallic acid fraction in accordance with residual solvent evaluation principles described in ICH Q3C(R8) and USP <467>.

V. FUTURE PERSPECTIVE

Further research should focus on the isolation, structural characterization, and pharmacological validation of novel bioactive compounds from *Mangifera indica* seed extracts. Toxicity studies, clinical investigations, and formulation development may facilitate their application in nutraceutical, pharmaceutical, and functional food industries.

REFERENCES

- [1] Harborne, J. B. (1998). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd ed. Chapman & Hall, London.
- [2] Jahurul, M.H.A., Zaidul, I.S.M., Ghafoor, K., et al. (2015). Mango (*Mangifera indica* L.) by-products and their valuable components: A review. *Food Chemistry*, 183, 173–180.
- [3] Maisuthisakul, P. and Gordon, M. H. (2014). Characterization and storage stability of the extract of Thai mango (*Mangifera indica* Linn. cultivar Chok-Anan) seed kernels. *J. Food Sci. Technol.* 51 : 1453-462.
- [4] Masibo, M., & He, Q. (2009). Mango Bioactive Compounds and Related Nutraceutical

Properties—A Review. Food Reviews International, 25(4), 346–370.

- [5] Mwaurah, P. W., Kumar, S., Kumar, N., Panghal, A., Attkan, A. K., Singh, V. K. and Garg, M. K. (2020). Physicochemical characteristics, bioactive compounds and industrial applications of mango kernel and its products: A review. Comp.
- [6] Ojha, P., Raut, S., Subedi, U. and Upadhya, N. (2019). Study of nutritional, phytochemicals and functional properties of mango kernel powder. J. Food Sci. Technol. Nepal. 11 : 32-38.
- [7] Quintana, S.E., Salas, S., & García-Zapateiro, L.A. (2021). Bioactive compounds of mango (*Mangifera indica*): A review of extraction technologies and chemical constituents. Journal of the Science of Food and Agriculture, 101, 6186–6192.
- [8] Ribeiro, S.M.R., Barbosa, L.C.A., Queiroz, J.H., et al. (2016). Mango seed: Functional and nutritional properties. Trends in Food Science & Technology, 55, 109–117.
- [9] Siller-Cepeda, J.A., et al. (2023). Bioactive Compounds in Extracts from the Agro-Industrial Waste of Mango. Molecules, 28(1), 458.
- [10] Skoog, D. A., Holler, F. J., & Crouch, S. R. (2018). Principles of Instrumental Analysis. 7th ed. Cengage Learning.
- [11] Soxhlet, F. (1879). Die gewichtsanalytische Bestimmung des Milchfettes. Dingler's Polytechnisches Journal, 232, 461–465.
- [12] Trease, G. E., & Evans, W. C. (2009). Trease and Evans Pharmacognosy. 16th ed. Saunders Elsevier.
- [13] Yadav, D., Pal, A.K., Singh, S.P., & Sati, K. (2022). Phytochemicals in mango (*Mangifera indica*) parts and their bioactivities: A Review. Agricultural Reviews, 57, 79–95.