ORIGINAL ARTICLE

High-resolution Mass Spectrometry Study of Palm Jaggery Components with an Emphasis on Anti-microbial Properties

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Abstract

Introduction: The palmyra (*Borassus flabellifer* L.) is a member of the Arecaceae family. The unfermented palmyra tree sap, or neera, is used to make jaggery. Antimicrobials are substances that kill or inhibit the growth of harmful microorganisms like bacteria, viruses, fungi, and parasites. The study focuses on elucidating the antimicrobial properties of palm jaggery by analyzing the bioactive metabolites identified through High-Resolution Accurate Mass Spectrometry (HRMS). **Methodology:** The optimised sample of Palm jaggery was tested for metabolomics analysis. The High-Resolution Accurate Mass Spectrometry system instrument with the model name Orbitrap Eclipse Tribrid Mass Spectrometer developed by Thermo Fisher Scientific was used for the analysis of Palm jaggery sample. The raw data obtained from the mass analyser were performed through default parameters of "Compound discoverer 3.3.2.31" using online databases. **Result:** Utilizing HRMS, we successfully identified a diverse array of bioactive compounds and other secondary metabolites. This study has identified four key metabolites namely Diselane, Selenophosphate, Cadmium Sulfide, and Carnosol as having notable antimicrobial properties. The reported antimicrobial efficacy of these components was systematically evaluated and previous studies revealed significant antibacterial activity, particularly against both Gram-positive and Gram-negative strains. **Conclusion:** Reported findings not only enhance the understanding of palm jaggery's biochemical profile but also highlight its significance as a source of antimicrobial agents, warranting further exploration for applications in food preservation and healthcare. This study contributes to the growing body of knowledge regarding the functional properties of traditional foods and their role in promoting public health.

Key words: Anti-microbial activity, *Borassus flabellifer*, High-resolution mass spectrometry analysis, Neera, Palm jaggery

INTRODUCTION

The palmyra (*Borassus flabellifer* L.) belongs to the ancient Arecaceae family and is commonly known as the sugar palm or toddy palm. This plant thrives in Southeast Asia and the Indian subcontinent and is easy to cultivate.[1] In Southern India, palm tree extract is used to produce palm jaggery, derived from the unfermented sap of the palmyra tree, known as *neera*. Due to its therapeutic properties, this product is relatively expensive and has a robust, earthy flavor reminiscent of chocolate. After processing, palm jaggery takes on a deeper, richer color and is valued for its cooling effects on the body. *Neera*, the liquid extracted from the tender fruit of *B. flabellifer* L., is rich in vitamins and minerals that enhance the body's defense mechanisms and boost immunity. It boasts a rich array of phytochemicals, anti-bacterial agents, and anti-oxidants, including Vitamin C, which collectively support overall health. *Neera* is particularly abundant in potassium, which promotes better blood circulation. In addition, its anti-bacterial and anti-oxidant properties contribute to better skin health and may help delay signs of aging. Regular consumption of *neera* can thus protect the skin from free radical damage that leads to oxidative stress.[1] Recently, there has been a surge of interest in discovering and developing novel anti-microbial compounds from diverse sources to combat microbial resistance. Anti-microbials, whether derived from natural

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sources or created synthetically, are substances that can kill or inhibit the growth of microorganisms. This includes agents that can suppress pathogen growth or act as microbicides. The need for novel anti-bacterial activity is driven by the urgent challenges posed by antibiotic resistance, the limitations of current treatments, and the potential for discovering new agents that can enhance public health and safety. Consequently, there has been a growing focus on techniques for screening and evaluating anti-microbial activity of natural compounds. Palm jaggery is recognized for its remarkable anti-bacterial and anti-oxidant properties.[2] As a result, efforts are made to isolate specific effective components from palm jaggery that exhibit anti-microbial properties.

MATERIALS AND METHODS

Preparation of palm jaggery

Palm jaggery is prepared from the sap of the palmyra palm. Initially, the sap is filtered and then heated to 120°C in a round–bottom pan on an induction stove. It is stirred continuously until it thickens and develops a rich brown color. This process continues until the syrup reaches a total soluble solids concentration of about 81° Brix. Once this concentration is achieved, the mixture is cooled, resulting in the final product: palm jaggery.[3]

Collection of palm jaggery

The raw material of Palm Jaggery for the analysis by High-resolution mass spectrometry was procured from Aranyaka e-Retail, 222/1, Kothukarar Thottam, Kumanan Nagar, Kumalankuttai, Erode-11. The same palm jaggery was later authenticated in the Department of Dravyaguna (related to Ayurvedic Pharmacognosy) with the accession number DG/24-25/852.

Method employed for HRMS analysis

Palm Jaggery, methanol, distilled water, and Eppendorf tube were used. The high-resolution accurate mass spectrometry system instrument was used with the model name Orbitrap Eclipse Tribrid Mass Spectrometer developed by Thermo Fisher Scientific. For small molecules, Dionex UltiMate 3000 RSUHPLC system was used for phytochemical analysis.[4]

The sample preparation for HRMS analysis started with the addition of the individual optimized sample of palm jaggery (100 mg) with 1.5 mL solvent (Methanol:water; 80:20) and homogenized using Eppendorf ThermoMixer at 750 rpm for 30 min at 25°C. Then, the sample was centrifuged (3500 rpm/10 min/25°C). The supernatant was filtered with a 0.22 µ PTEF syringe filter and 4 µL of the filtrate was used as injection volume on C18 RP-HPLC column (Hypersil GOLD™: Particle size 1.9 µ, 2.1 mm × 100 mm).

The reversed-phase chromatographic separation starts with a high aqueous phase (+0.1% formic acid) and ends on highly organic phase (MeOH+ 0.1% formic acid) typically 100% aqueous to 100% organic. The LC gradient parameters were 0–6 min 5% MeOH, 6–10 min 30% MeOH, 10–20 min 50% MeOH, 20–25 min 90% MeOH, 25–27 min 90% MeOH, and 27–30 min 5% with flow rate of 300 L/min and column oven temperature 40°C.

The optimized sample of Palm jaggery was tested for metabolomics analysis. Thermo Fisher Scientific – High-resolution accurate mass spectrometry system of the model "Orbitrap EclipseTribrid Mass Spectrometer coupled with Nano Liquid Chromatography and Ultra High-pressure liquid chromatography" (Diones Ultimate 3000 RSLC) system, Heated Electro Spray Ionisation source was used to fed the sample to the mass spectrometer post-chromatographic separation. The Orbitrap analyzer was utilized at 60,000 resolutions separately for positive/negative polarity with mass range (m/z) 100–1000, 35% RF Lens, 25% normalized AGC target keeping 2.0e5 as intensity threshold to perform MS-OT (Master scan). To obtain ddMS2 OT HCD the selection parameters were, quadrupole isolation mode with 1.5 isolation window (m/z) HCD activation type, 30, 45, 60HCD collision energy (%), 15000 orbitrap resolution, and 20% normalized AGC target.[5]

The raw data obtained from the mass analyzer were performed through default parameters of "Compound discoverer 3.3.2.31" using online databases. The selected workflow was the Natural Product Unknown ID method, leveraging both online and local database searches. This untargeted food research approach focuses on detecting and identifying unknown compounds without statistical analysis. It aligns retention times, detects and groups unknown compounds across all samples, and predicts elemental compositions for each. Blank samples were used to hide the chemical background. Compound identification is achieved through mzCloud (ddMS2 and/or DIA), ChemSpider (using exact mass or formula), and local database searches against mass lists (with or without retention time). For compounds with ddMS2 data, mzCloud is used for spectral similarity searches, whereas spectral distance scoring is applied to ChemSpider and mass list matches.[6]

RESULTS

A total ion chromatogram (TIC) represents the overall intensity of all ions detected over time, providing a crucial tool for interpreting a sample's composition and identifying the compounds within it. As a visual display of the ions detected during chromatographic separation, the TIC offers valuable insights into the sample's makeup. The TIC of the components in palm jaggery is shown in Figure 1.

A standard ion chromatogram serves as a reference for identifying and quantifying ions in the test sample. This allows for precise determination of the bioactive compounds present

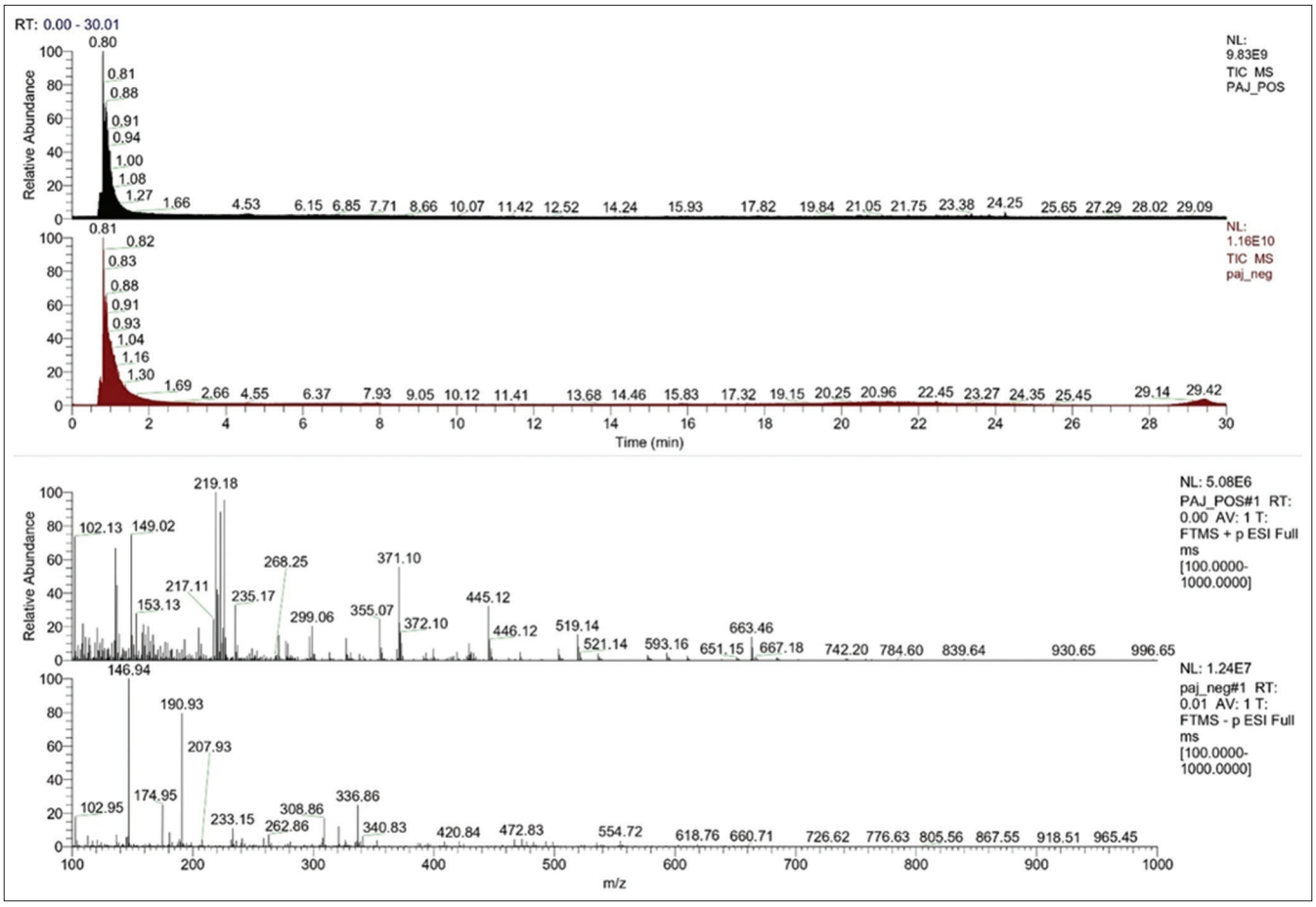

Figure 1: Depicts the total ion chromatogram obtained from UHPLC-HRAMS analysis of the *Borassus flabellifer* (palm jaggery) sample in both positive and negative ion modes

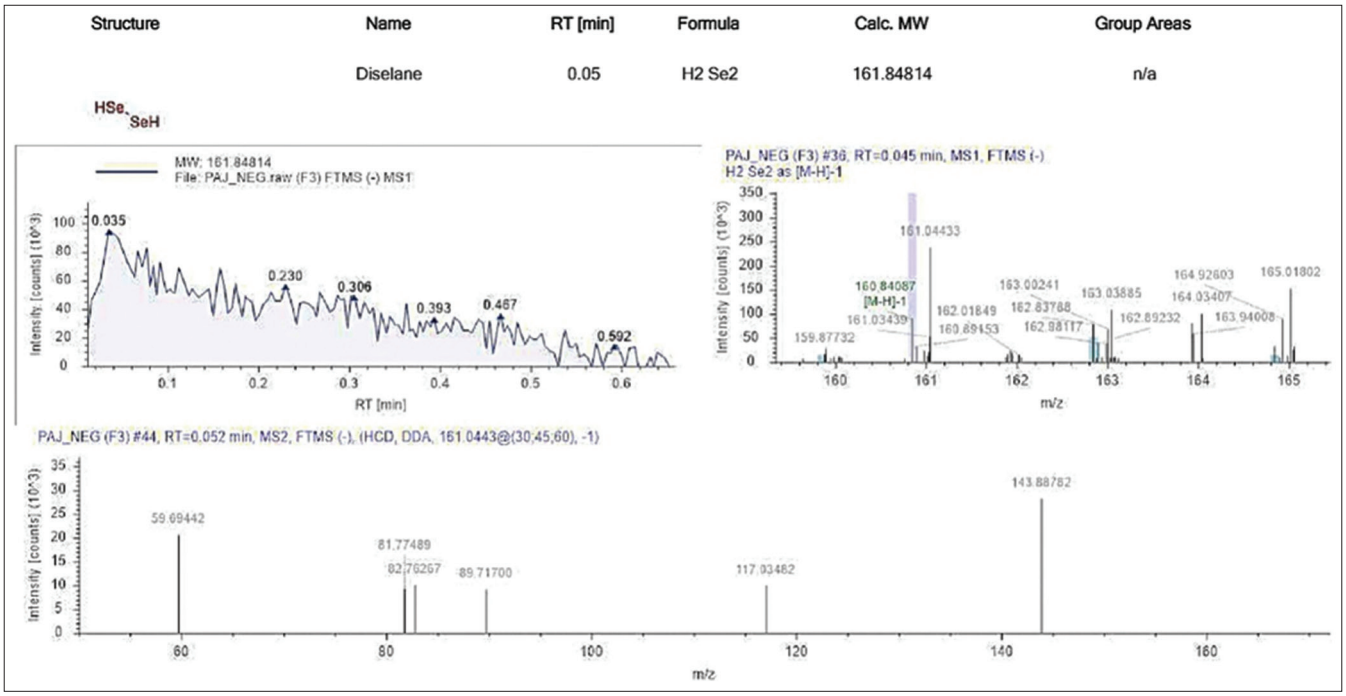

Figure 2: Standard ion chromatogram of the component diselane isolated from *Borassus flabellifer* (palm jaggery)

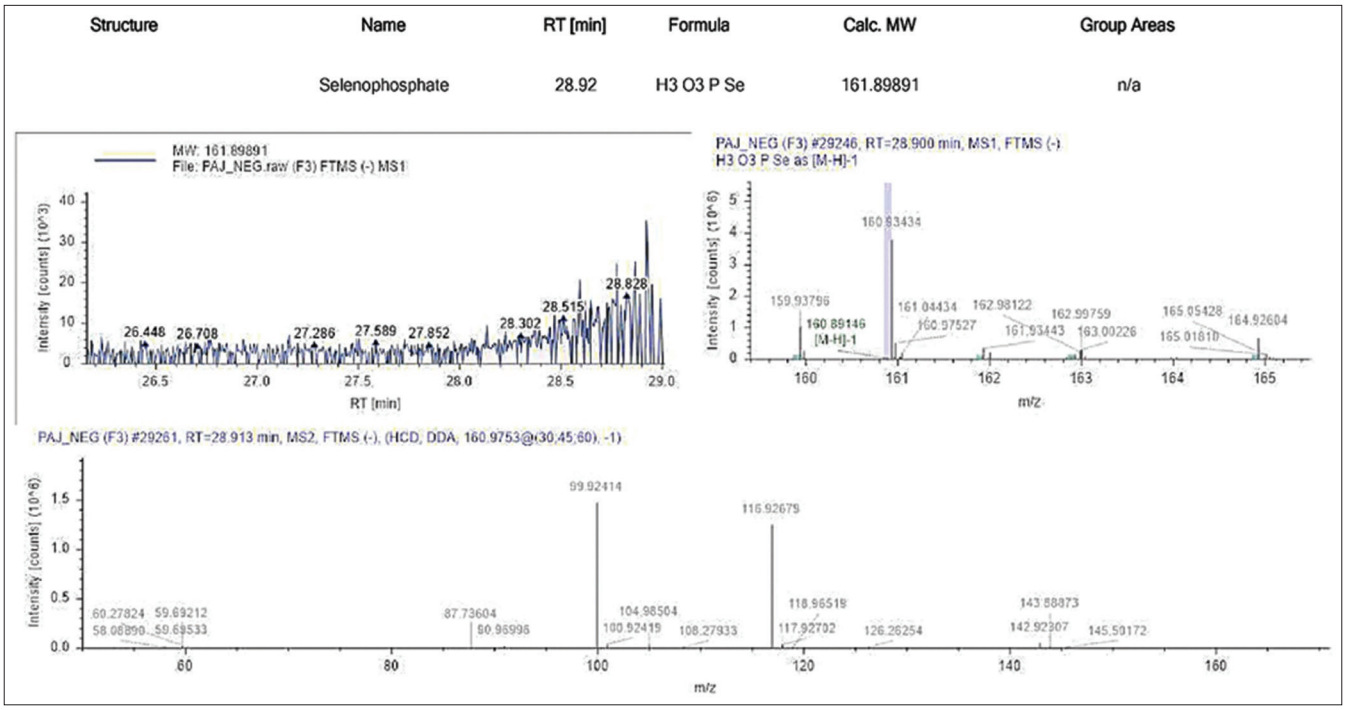

Figure 3: Shows standard ion chromatogram of the component selenophosphate isolated from *Borassus flabellifer* (palm jaggery)

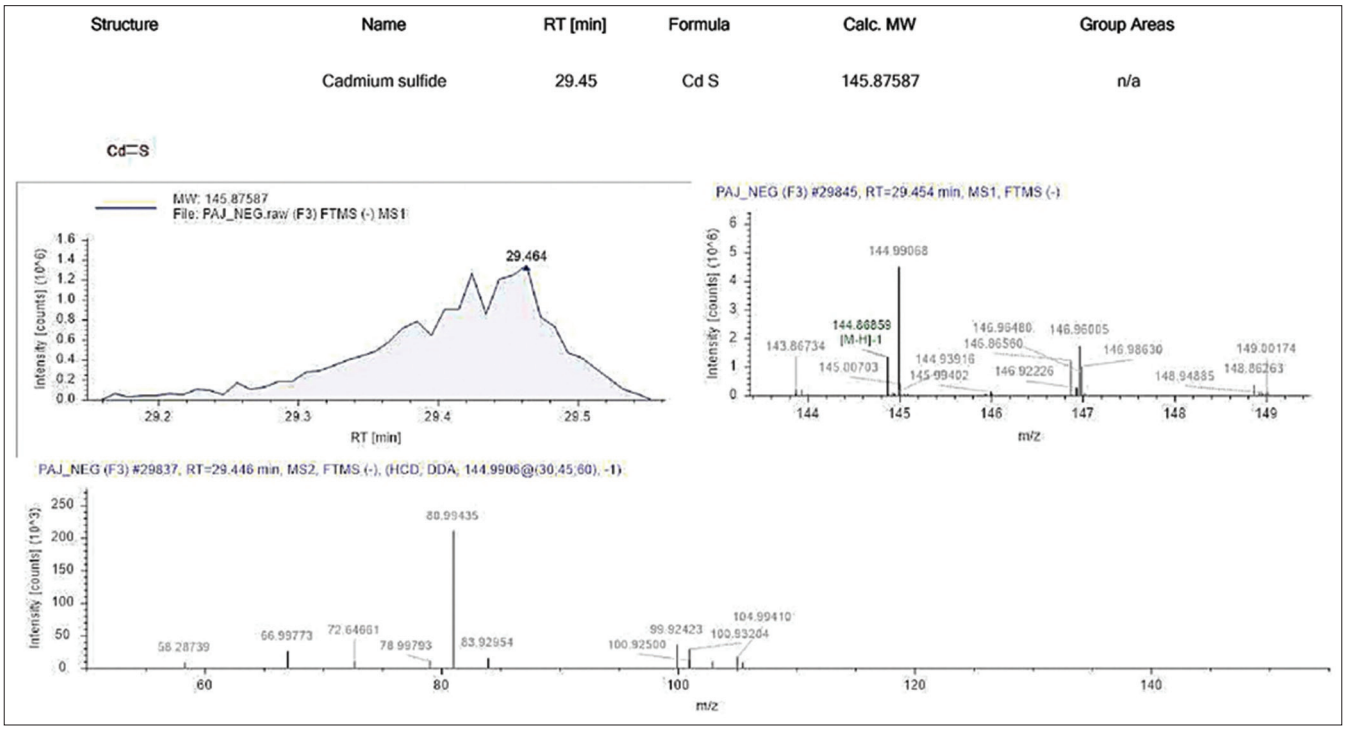

Figure 4: Standard ion chromatogram of the component cadmium sulfide isolated from *Borassus flabellifer* (palm jaggery)

in the sample. The anti-microbial components/ions identified in Palm Jaggery were quantified and characterized based on their retention times and peak intensities, as illustrated in Figures 2–5.

Anti-microbial properties of the bioactive components present in Palm Jaggery:

Using HRMS analysis, a total of 366 phytochemical constituents were identified in the Palm Jaggery sample. Notably, the following components demonstrate anti-bacterial activity according to the cited references: (1) diselane; (2) selenophosphate; (3) Cadmium sulfide, and (4) carnosol. These compounds are significant for their potential role in the anti-microbial properties of Palm Jaggery.

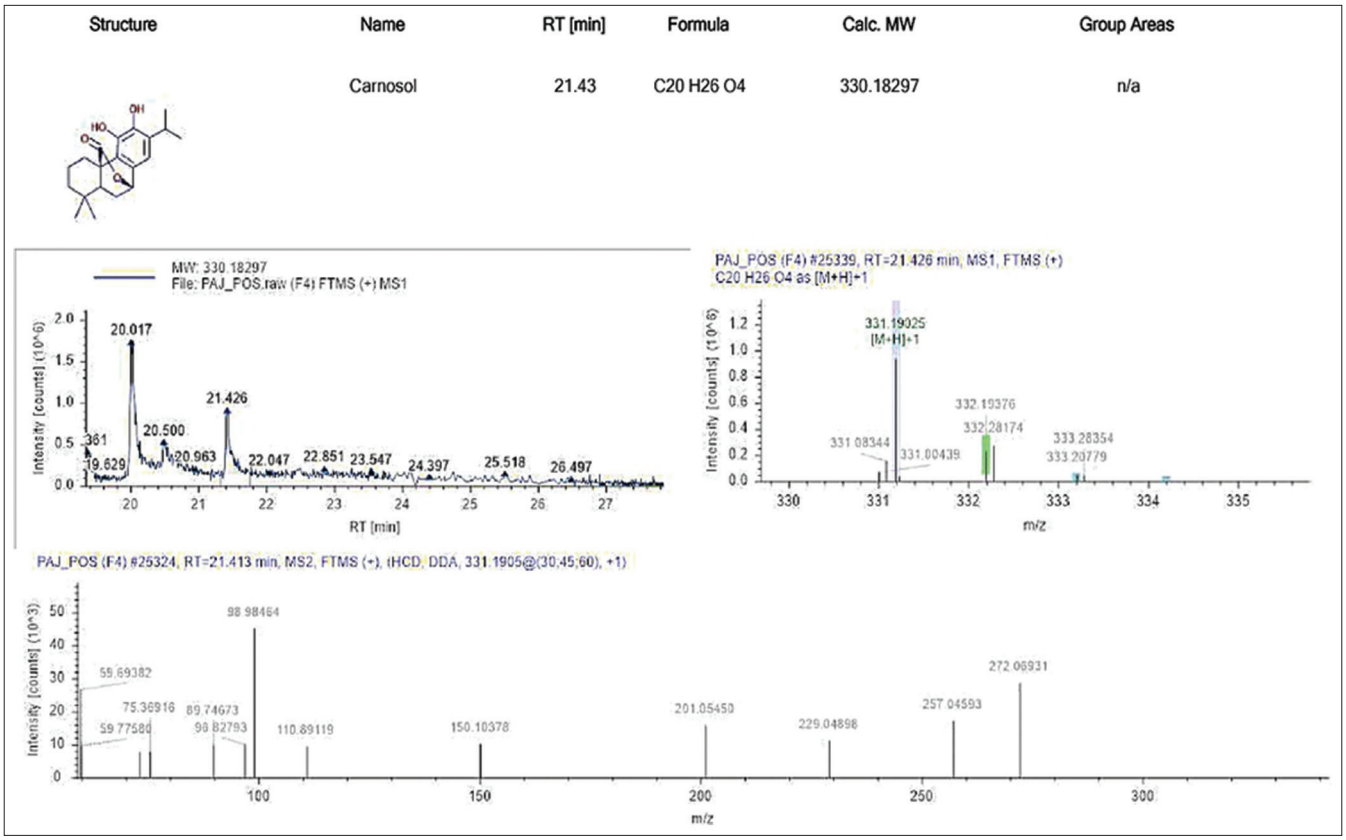

Figure 5: Standard ion chromatogram of the component carnosol isolated from *Borassus flabellifer* (palm jaggery)

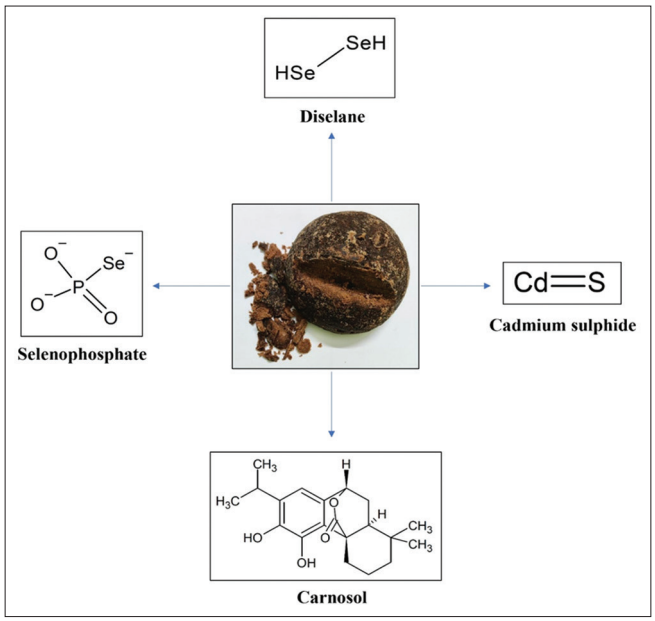

Figure 6: Molecular structure of isolated phytochemical constituents obtained from *Borassus flabellifer* (palm jaggery) possessing therapeutic effect against various micro-organisms by high-resolution mass spectrometry analysis

Diselane

1,2-Di(quinazolin-4-yl)diselane (DQYD), a derivative of quinazoline, shows promising anti-mycobacterial property. DQYD exhibits a low minimum inhibitory concentration (MIC) and demonstrates dose-dependent bactericidal activity, effectively inhibiting mycobacterial cultures within 8–12 days. Its efficacy against *Mycobacterium tuberculosis* appears linked to the maintenance of intracellular ATP homeostasis and the levels of DNA damage. Notably, there is no observed correlation between the survival of mycobacteria in the presence of DQYD and the levels of reactive oxygen species or iron. These findings indicate that DQYD could be a novel anti-mycobacterial drug with bacteriostatic properties, although the underlying mechanisms warrant further investigation.[7] The standard ion chromatogram of Diselane is depicted in Figure 2.

Selenophosphate

As per previous reported studies, the anti-microbial activity of selenium nanoparticles (SeNPs), both alone and in combination with standard antibiotics, has been evaluated against a variety of microorganisms, including Gram-negative and Gram-positive bacteria, as well as fungi. However, there is limited research on the specific mechanisms underlying the antimicrobial effects of these nanoparticles. Generally, it is suggested that SeNPs may exert their antimicrobial action through three primary mechanisms: (i) cell wall and membrane damage, (ii) intracellular penetration, and (iii) induction of oxidative stress.[8] The standard ion chromatogram of Selenophosphate is displayed in Figure 3.

Cadmium sulfide

Previous studies have demonstrated that synthesized cadmium sulfide nanoparticles (CdS NPs) possess notable antimicrobial properties. The sensitivity of microorganisms to CdS NPs correlates directly with their concentration; higher concentrations result in a greater number of CdS NPs interacting with bacterial cells, thereby increasing the likelihood of anti-microbial action. Both Gram-positive and Gram-negative bacteria exhibited susceptibility to the synthesized CdS NPs, with the zone of inhibition expanding in response to increasing nanoparticle concentration.[9] Certain studies have investigated the anti-microbial activity of cadmium nanoparticles (CdNPs) against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, and *Candida albicans*. The findings indicate that CdNPs exert a stronger effect on Gram-positive bacteria compared to Gram-negative bacteria, as evidenced by the size of the inhibition zones. CdNPs demonstrated significant anti-bacterial effects against all tested bacterial pathogens. This variation in microbial susceptibility may be attributed to the differences in cell wall structure, which influences the permeability of microorganisms.[10] The standard ion chromatogram of Cadmium sulfide is illustrated in Figure 4.

Carnosol

It is reported that carnosol exhibits significant inhibitory effects against both yeasts and various bacteria, establishing itself as the most potent anti-microbial agent, especially at a concentration of 150 µg/mL. At lower concentrations, it proved to be more effective against bacteria compared to the other tested compounds. Overall, carnosol was a superior inhibitor of microbial growth in laboratory media when compared to butylated hydroxytoluene and butylated hydroxyanisole.[11] Another study reported that the anti-bacterial efficiency is notably enhanced by the presence of carnosol, the primary diterpene compound. Increasing the concentration of carnosol significantly boosts anti-bacterial activity.[12] Carnosol is reported to possess broad anti-microbial activity against oral pathogens. Studies indicate that carnosol may disrupt the cell membrane of *C. albicans*, the primary cause of oral thrush.[13] It is also reported that carnosol also exhibits anti-bacterial activity against oral bacterial pathogens, including *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus sobrinus*, *Streptococcus mitis*, *Streptococcus sanguinis*, and *Enterococcus faecalis*, with MICs ranging from 35 to 100 mg/mL.[14] The standard ion chromatogram of carnosol is depicted in Figure 5.

DISCUSSION

Natural compounds derived from plants, fungi, and other organisms have gained considerable attention for their antimicrobial properties. Natural anti-microbials can act through various mechanisms, such as disrupting microbial cell membranes, inhibiting nucleic acid synthesis, and interfering with metabolic pathways. Unlike synthetic antibiotics, natural compounds often have complex structures and varied mechanisms of action, which may reduce the likelihood of developing resistance in microorganisms. When combined with conventional antibiotics, natural compounds can enhance anti-microbial efficacy, offering a promising strategy to combat resistant strains. This synergy can lower the required doses of antibiotics, minimizing side effects. Ayurveda, the traditional Indian system of medicine, has long recognized the anti-microbial properties of various natural substances. Ayurveda offers a rich repository of natural compounds with anti-microbial activity, emphasizing holistic health, and preventive care. Palm jaggery could be classified under the Guda Kalpana mentioned in the ayurvedic treatises. Palm jaggery, a natural traditional unrefined sugar made from the sap of the palmyra palm, is not only valued for its nutritional benefits but also for its anti-microbial properties.

Palm jaggery contains various bioactive compounds which contribute to its ability to inhibit microbial growth. Research indicates that palm jaggery exhibits significant anti-bacterial activity against a range of pathogens, including both Gram-positive and Gram-negative bacteria. In various cultures, palm jaggery has been traditionally used not just as a sweetener but also for its health benefits, including its ability to boost immunity and promote digestive health, which are often linked to its anti-microbial properties. Hence, an effort has been made to highlight the anti-microbial potential of Palm jaggery by conducting high-resolution mass spectrometry (HRMS) analysis to identify and isolate the active compounds that may contribute to its anti-microbial effects. The optimized Palm jaggery sample was subjected to metabolomics analysis using HRMS. The raw data from the mass spectrometer were analyzed with default parameters in "Compound Discoverer 3.3.2.31," utilizing online databases for comprehensive evaluation. HRMS analysis revealed 366 phytochemical constituents in the Palm jaggery sample. Among those phytochemical components, the following bioactive metabolites, namely: Diselane; Selenophosphate; Cadmium Sulfide; and Carnosol are reported to demonstrate anti-bacterial activity. The brief activity of each identified metabolite is mentioned in Table 1.

The anti-microbial activity of the compound DQYD against *M. tuberculosis* operates through mechanisms involving intracellular ATP homeostasis and DNA damage.[7] SeNPs exhibit their anti-microbial effects through several pathways like damaging the cell wall and membrane; penetrating the cell, and by inducing oxidative stress.[8] Cadmium sulfide displays anti-microbial properties primarily by causing damage to the cell wall and membrane.[10] Carnosol demonstrates anti-microbial properties by disrupting the bacterial cell membrane.[13] A summarized overview of the metabolites with identified anti-microbial activity is presented in Table 2.

Table 1: Phytochemical components isolated from *Borassus flabellifer* (palm jaggery) by high-resolution mass spectrometry technique with their antibacterial activity on different micro-organisms

Sr. No.	Phytochemical Constituents	Effective against
1.	Diselane	*Mycobacterium tuberculosis*
2.	Selenophosphate	a wide range of microorganisms, including Gram-negative, Gram-positive bacteria, and fungi
3.	Cadmium sulfide	*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, and *Candida albicans*; has greater effect on the gram-positive bacteria than on the gram-negative bacteria
4.	Carnosol	*Candida albicans*, *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus sobrinus*, *Streptococcus mitis*, *Streptococcus sanguinis*, *Enterococcus faecalis* and yeasts

Table 2: Depicts the details of the metabolites isolated by high resolution mass spectrometry. It shows the chemical formula, calculated MW, RT values, delta mass, and the peak area under NEG/POS ion mode of the bioactive metabolites

Bioactive metabolite	Formula	Calc. MW	Retention time (min)	Delta mass (ppm)	Area NEG	Area POS
Diselane	H_2Se_2	161.84814	0.045	−3.4	1385197.32	-
Selenophosphate	H_3O_3PSe	161.89891	28.918	2.51	556784.37	-
Cadmium sulfide	CdS	145.87587	29.452	3.01	10076016.36	-
Carnosol	$C_{20}H_{26}O_4$	330.18297	21.429	−0.42	-	7680117.24

RT: Retention time, MW: Molecular weight, NEG: Negative, POS: Positive

The chemical structure of anti-microbial compounds significantly influences their efficacy through factors such as functional groups, hydrophobicity, molecular size and shape, charge, and stability. These attributes determine how well a compound can penetrate microbial membranes, interact with cellular targets, and exert its action, whether by disrupting cell walls or DNA. In addition, understanding the structure-activity relationship (SAR) allows for the design of more potent agents. Hence, the molecular structure of the active components found in Palm jaggery is depicted in Figure 6.

This study offers important insights into the chemical constituents and anti-microbial properties of palm jaggery, but it has several limitations. First, while we identified various bioactive compounds, the specific mechanisms underlying their anti-microbial action were not explored and warrant further investigation. Second, the anti-microbial efficacy reported in this study is based on the particulars that were assessed in laboratory settings, which may not accurately reflect real-world conditions for food preservation or therapeutic applications. In addition, the potential effects of processing methods and environmental factors on the bioactivity of palm jaggery were not considered. Finally, further research is needed to evaluate the safety and effectiveness of these compounds *in vivo* and to investigate their interactions with other food ingredients or pharmaceuticals.

CONCLUSION

In summary, this study underscores the rich array of chemical constituents in palm jaggery and their notable antimicrobial properties, as demonstrated through HRMS. The identification of various bioactive compounds positions palm jaggery as a promising natural source of anti-microbial agents, effective against both Gram-positive and Gram-negative bacteria. These results not only deepen our understanding of palm jaggery's biochemical profile but also highlight its potential applications in food preservation and healthcare. Nonetheless, further research is needed to clarify the mechanisms of action, assess efficacy in real-world scenarios, and evaluate the safety of these compounds in diverse applications. Overall, this study adds to the expanding knowledge of traditional foods and their potential contributions to public health.

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