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ORIGINAL ARTICLE

Evaluation of *Arka* and *Arista* Extracts from *Woodfordia fruticosa* Kurz (*Dhathaki*) flowers as antimicrobial agents: A Gas Chromatography-Mass Spectrometry Study

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ABSTRACT
This study is an explorative work on the chemical composition of formulations prepared using the flowers of Woodfordia fruticose (Dhathaki). Three formulations/extracts have been prepared to achieve this objective. The chemical composition was studied with the intent to decipher the presence of antimicrobial compounds in the extracts. The three extracts prepared include an aqueous extract (Dhathaki-Aq), a distilled extract (Arka; Dhathaki-A), and a fermented preparation (Arista; Dhathaki-AA). All three extracts were subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis to determine the bioactive compounds with antimicrobial activity in the extracts. The GC-MS results showed that Arka and Arista had high levels of ethanol (over 90%), a compound known for its antimicrobial properties, with few impurities. On the other hand, the aqueous extract had a complex mixture of volatile organic compounds with no dominant antimicrobial compound detected. This suggests the need for further purification or optimization of aqueous extract preparation to investigate bioactive components. Overall, the findings highlight the antimicrobial potential of Arka and Arista, and their possible use as antimicrobial products such as hand sanitizers. However, additional experimental confirmation for microbicidal activity, skin safety, and toxicity are needed to ensure their effectiveness and safety for human use. Also, the Arka and Arista formulations can further be upgraded with the addition of other antimicrobial agents to enhance their effectiveness. These results obtained with the successful application of modern scientific methods in the traditional Ayurveda opens up new opportunities for natural therapeutic and antimicrobial products.

Keywords: *Woodfordia fruticosa, Arka, Arista, Gas Chromatography-Mass Spectrometry (GC-MS), antimicrobial properties, alcohol*

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INTRODUCTION

Medicinal plants have been an integral part of traditional healthcare systems, providing therapeutic agents for centuries [1]. *Woodfordia fruticosa* Kurz (*Dhathaki*) is one such medicinal plant extensively used in Ayurvedic medicine as it possesses a wide range of pharmacological effects. This plant is native to the Indian subcontinent and Southeast Asia and has been used for medicinal purposes for a long time. Different parts of this plant have distinct medicinal benefits. For instance, the roots have astringent and anti-inflammatory properties, the leaves aid in wound healing and possess antimicrobial effects, and the

bark is effective in the treatment of diarrhoea and dysentery. However, the flowers of *Dhathaki* are most targeted for their medicinal value [2-4]. They play a key role in fermentation by serving as a natural catalyst during the preparation of Ayurvedic remedies like *Arista* and *Asava* [5, 6]. They are rich in tannins, flavonoids, and other phytochemicals and thus exhibit strong antimicrobial, antioxidant, and immunomodulatory properties. These compounds are known to enhance the therapeutic effects of the *Dhathaki* flower-based formulations and help preserve them by inhibiting microbial growth [6].

Arka, a distilled preparation, and *Arista Arka*, a fermented herbal formulation, are two distinct Ayurvedic dosage forms. *Arka* is prepared by distillation, enabling the extraction of volatile and water-soluble components from the plant material to create a concentrated formulation [7]. In contrast, *Arista Arka* is produced through fermentation, enriching the formulation with bioavailable metabolites that are generated by microbial activity (Panda et al. 2022). Despite their long history of use in traditional medicine, there is limited scientific data on their chemical composition and antimicrobial properties. Addressing this gap is vital to scientifically examine the effectiveness of traditional knowledge in tackling modern healthcare issues (eg. antibiotic resistance).

Gas Chromatography-Mass Spectrometry (GC-MS) is a sophisticated technique that enables the identification and quantification of volatile and semi-volatile compounds in a test sample. It is beneficial for detecting bioactive molecules with medicinal properties and thus can be applied in determining the phytochemical components responsible for the therapeutic effects of traditional remedies too [8-10]. With the approach of applying GC-MS in Ayurvedic studies, the gap between traditional practices and modern science can be bridged. This enables the integration of ancient knowledge into contemporary healthcare solutions.

In this study, extracts from the flowers of *W. fruticosa* Kurz were processed into *Arka* (*Dhathaki*-A) and *Arista Arka* (*Dhathaki* AA) using traditional Ayurvedic methods. These formulations were analyzed using GC-MS to identify antimicrobial agents and assess their potential therapeutic applications. This investigation aims to scientifically validate the antimicrobial properties of the aforementioned preparations. By detailing the phytochemical profile and bioactivity of these formulations, the study seeks to enhance our understanding of *Dhathaki*'s role in traditional and modern medicine, emphasizing its potential to address therapeutic challenges.

MATERIAL AND METHODS

Collection of *Dhathaki Pushpa*

Dhathaki Pushpa (flowers of *W. fruticosa* Kurz) were procured from Annamaya Herbals, KM Marga, Udupi. Upon procurement, the flowers were carefully transported to the Department of RSBK, SDMCAH, Hassan, Karnataka, under appropriate conditions to prevent any damage or contamination. The flowers were pulverized, and the resultant powder (*Dhathaki Pushpa choorna*) was stored in a cool and dry place until future use.

Preparation of Aqueous Extract from *Dhathaki Pushpa* (*Dhathaki*-Aq)

Cold maceration was employed to prepare the aqueous extract of *Dhathaki* dry flowers (*Dhathaki*-Aq). Briefly, a 100 g portion of *Dhathaki Pushpa choorna* was added to 300 mL of distilled water in a 1000 mL conical flask. The flask was placed on a rotator shaker and shaken for 10-15 min at regular intervals of 3 hr. This process was repeated daily for 7 days to ensure efficient extraction of the soluble components from the flowers. After 7 days, the contents of the conical flasks were filtered to obtain the aqueous extract [11]. The clear aqueous filtrate thus collected was subsequently used for preparation of *Dhathaki*-A and *Dhathaki* AA.

Preparation of *Arka* from *Dhathaki Pushpa* (*Dhathaki*-A)

About 170 g of *Dhathaki Pushpa choorna* was soaked in 340 mL of water overnight to facilitate the initial extraction. Another 510 mL of water was added the following day, and the mixture was distilled to extract the volatile components. To achieve this, an *Arka Yantra* at 88 °C for 3 hr. After distillation, 510 mL (60%) of *Arka* (hydrosol) was obtained, yielding a 1:5 ratio of *Arka* - a concentrated extraction of essential components [12]. The collected *Arka* (*Dhathaki*-A) was transferred into sterile 30 mL bottles and appropriately labelled for future use.

Preparation of *Arista* from *Dhathaki Pushpa* (*Dhathaki*-AA)

A 188.87 g of *Dhathaki Pushpa choorna* was added to 2000 mL of water in a stainless steel vessel. The mixture was heated on *mridu agni* (low heat) to bring down its volume to 1/4th of the original volume i.e., 500 mL. Later, it was filtered and allowed to cool. Once cooled, 195 g of jaggery was dissolved to prepare a decoction (*kashaya*), to which 20 g of *Dhathaki Pushpa choorna* and 98 g of *Madhu* (honey) were added. The resulting mixture of *kashaya*, termed *prakshepaka*, and honey was transferred to a container, sealed with *sandhi bandhana* using *multani mitti* (Fuller's earth), and placed in a husk for fermentation over one

month. After one month, the *sandhi bandhana* was removed, and the mixture was subjected to a candle test, which returned negative, indicating insufficient alcohol production despite the faint presence of an alcoholic odour. The container was re-sealed, and allowed to ferment for an additional week. After this duration, upon opening the *sandhi bandhana*, it was observed that the *prakshepaka dravya* had fully sunk. The candle test was now positive, confirming the presence of alcohol [13]. The fermented *Dhathaki Arista* was then subjected to distillation. The initial distillate drops were discarded, and 60% of the *Arka* (distillate) was then collected. The remaining substrate was discarded. The collected *Arista Arka* was then transferred into sterile 30 mL bottles and appropriately labelled for future use.

GC-MS Analysis of Aqueous Extract of *Dhathaki*, *Dhathaki Arista*, and *Dhathaki Arka*

All three extracts of *Dhathaki Pushpa*, namely *Dhathaki*-Aq, *Dhathaki*-A, and *Dhathaki*-AA were analyzed using a GCMS-QP2010 SE system equipped with a DB 5MS column (30 m x 0.250 mm diameter x 0.25 µm thickness) to identify bioactive compounds present in the samples. The injection port was set to 280 °C, and the analysis was carried out in split injection mode with a split ratio of 10 to control the sample amount introduced into the column. Helium was used as the carrier gas, flowing at a rate of 1.5 mL/min, ensuring efficient separation of compounds in the column. The oven temperature was initially set to 80 °C, held for 2 min., and then ramped to 280 °C at 10 °C/min for 10 min., followed by a further increase to 330 °C at 20 °C/min, which was maintained for 5 min. Mass spectrometric analysis was conducted using an electron ionization system with an ion source temperature of 200 °C and an interface temperature of 300 °C. Data acquisition was performed in scan mode, scanning the mass range of 35–500 m/z with a scan speed of 1666 scans/sec and an event time of 0.3 sec. A solvent cut time of 1.40 min. was used to eliminate the solvent interference from the spectra. Compounds were identified based on their retention times and mass spectra, which were compared to the NIST library for accurate compound identification [14]. This comprehensive GC-MS approach facilitated the identification and profiling of bioactive compounds in the *Dhathaki*-Aq and its derivatives.

RESULTS AND DISCUSSION

Preparation and Yield of Aqueous Extract, Arka, and Arista from Dhathaki Pushpa The preparation of three extracts from Dhathaki Pushpa namely Dhathaki-Aq, Dhathaki-A, and Dhathaki-AA was accomplished and prepared for additional examination. To explain, the cold maceration of Dhathaki flowers resulted in 210 mL of clear aqueous extract (Dhathaki-Aq). The distillation of Dhathaki Pushpa resulted in 510 mL of Arka (hydrosol), indicating concentrated extraction of essential components in Dhathaki-A. The preparation of Dhathaki Arista involved a fermentation process leading to alcohol production, as confirmed by the candle test. This fermented mixture was subsequently subjected to distillation resulting in the collection of 60% Arka (Dhathaki-AA). These three extracts were appropriately labelled and stored for future use.

Ayurvedic preparations such as Arka and Arista are known to possess therapeutic properties including antimicrobial, antioxidant, and immunomodulatory effects. In the process of Arka preparation, the volatile compounds present in a herbal plant are distilled into the extract. On the other hand, in the case of Arista, the volatile compounds are enriched via fermentation. Though these preparations are extensively used in Ayurvedic practice, chemical composition or information on the potential compound they contain are insufficiently studied. This study is an attempt to address this gap by bridging traditional practices with modern science.

Chromatographic Analysis of the Aqueous Extract: Compound Profile and Composition

In the GC-MS analysis of the Dhathaki-Aq, the extract was found to be a highly complex mixture as a total of 121 peaks were recorded in the chromatogram. The retention times of the compounds present in the extract ranged from 5.778 to 73.751 min. Of the 121 peaks, only a few were prominent and a majority were minor peaks, thus indicating that the compounds in the extract were of varying concentrations. The most significant peak was recorded at a retention time of 12.595 min. with a total area of the peak was 26.2%. Other significant peaks were observed at retention times of 6.435 min. (7.41%), 6.753 min. (7.45%), and 17.694 min. (9.3%). The minor peaks contributed to less than 1% of the total area indicating the presence of trace compounds or impurities in the extract. Overall, the peaks were broadly distributed, suggesting a diverse chemical profile most likely composed of volatile organic compounds (VOCs), and water-soluble metabolites (Figure 1).

The aqueous extract, while complex and chemically diverse, lacked ethanol or other well-known antimicrobial agents in dominant concentrations. This suggests that any potential therapeutic effects may be attributed to other VOCs or bioactive compounds. A compound at 12.595 min. stood out with over 25% of the area, potentially indicating a significant bioactive molecule. Further purification and optimization of the extraction process are necessary to enhance the identification of such key compounds.

Chromatographic Analysis of Dhathaki Arka: Dominance of Ethanol and Minor Impurities

In the GC-MS analysis of Dhathaki-A, ethanol was found to be the predominant compound with trace amounts of ethyl ether, trimmethylethylene, methylene chloride, and trichloromethane. The peak representing ethanol in the chromatogram of the extract showed total area and peak height of 91.86% and 66.96%, respectively. This accounted for an Area/Height (A/H) ratio of 11.66. The other compounds were recorded in trace amounts ranging from 0.08 - 0.14%. These compounds may likely be residual solvents or impurities in the extract. To summarize, Dhathaki-A preparation predominantly contained ethanol with other trace compounds (Figure 2).

The presence of ethanol in over 90% concentration indicates a potent antimicrobial potential, as ethanol is widely used for such purposes. Additionally, the presence of only trace impurities such as trichloromethane and methylene chloride suggests a high level of refinement in the preparation. Previous findings by our group support the antimicrobial application of Dhathaki Arka, specifically as a hand sanitizer. However, to validate its safety and effectiveness in human applications, further studies including skin toxicity assessments are warranted.

Chromatographic Analysis of Dhathaki Arista from Arka:

Predominance of Ethanol and Trace Impurities In the GC-MS analysis of Dhathaki-AA too, ethanol was found to be the predominant compound. In the chromatogram, the corresponding peak showed a total area and peak height of 92.71% and an A/H ratio of 11.61 (Figure 3). Besides ethanol, other compounds including acetone, cyclopropane, cyclohexane, methylene chloride, trichloromethane, and carbon tetrachloride were recorded in trace amounts (ranging from 0.10 - 0.18%). These compounds may likely be residual solvents or impurities in the extract. To summarize, the primary constituent present in Dhathaki-AA extract was ethanol.

The slightly higher ethanol content in Dhathaki-AA compared to Dhathaki-A suggests a stronger potential antimicrobial efficacy. Ethanol's effectiveness in concentrations above 60% is well-documented. Therefore, the high purity of Dhathaki-AA with minimal impurities enhances its candidacy for use in therapeutic and antimicrobial formulations. Nevertheless, safety evaluations must precede clinical use.

Comparative Analysis of GC-MS Profile of the Extracts

Upon comparison of GC-MS data of all three extracts, ethanol, a known antimicrobial agent, was found to be the dominant compound in Dhathaki-A and Dhathaki-AA. In contrast, Dhathaki-Aq extract was a complex mixture of VOCs and trace amounts of impurities/residual solvents without a clear dominant antimicrobial agent. Among Dhathaki-A and Dhathaki-AA, ethanol concentration was slightly higher in the Arista (AA) indicating that it could possess a better antimicrobial efficacy than the Arka (A).

The comparative analysis highlights a clear distinction: while Arka and Arista are ethanol-rich and hence more suitable for antimicrobial applications, the aqueous extract presents a complex yet promising chemical profile that needs further fractionation to identify active components. The presence of VOCs and the absence of ethanol in Dhathaki-Aq reinforce the idea that alternative bioactive constituents could be responsible for therapeutic effects. Future work should focus on refining the extraction processes, conducting bioactivity assays, and developing formulations that harness the distinct advantages of each extract type. Additionally, combining ethanol-based extracts with known antimicrobial agents such as tea tree oil or phenolic compounds could further improve their efficacy.

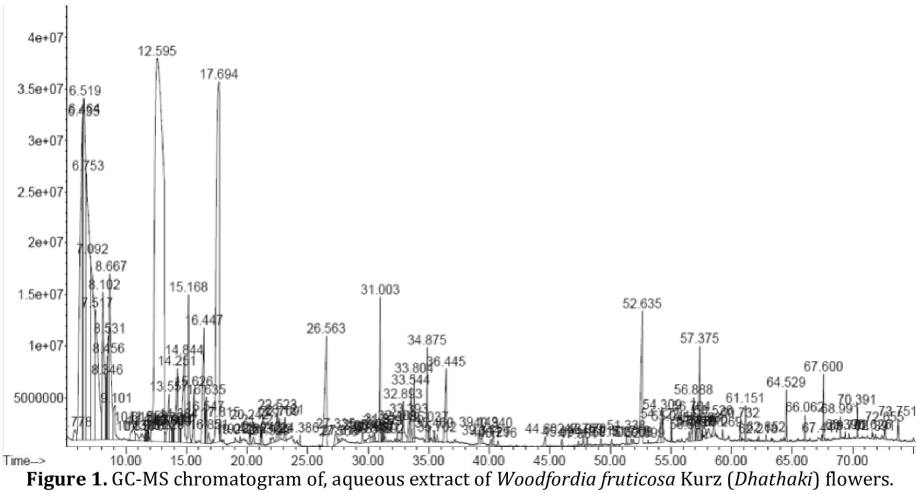


Figure 1. GC-MS chromatogram of, aqueous extract of *Woodfordia fruticosa* Kurz (*Dhathaki*) flowers.

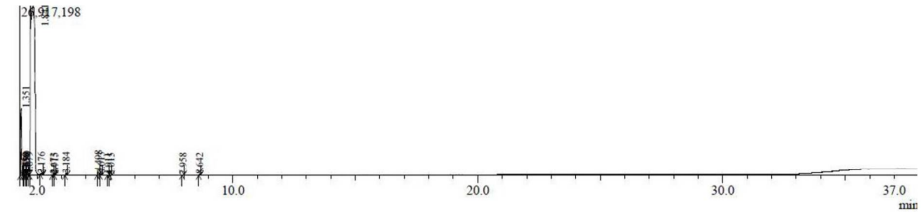


Figure 2. GC-MS chromatogram of, *Arka* extract of *Woodfordia fruticosa* Kurz (*Dhathaki*) flowers.

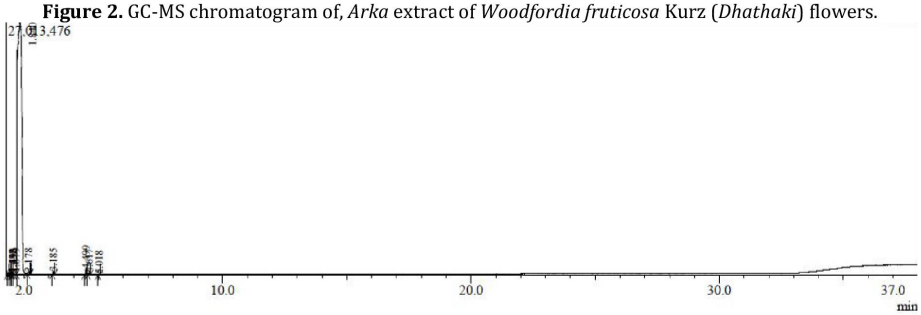
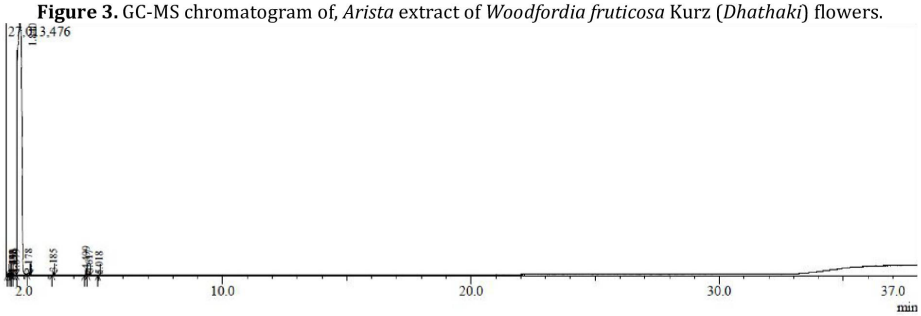


Figure 3. GC-MS chromatogram of, *Arista* extract of *Woodfordia fruticosa* Kurz (*Dhathaki*) flowers.



CONCLUSION

In conclusion, in this study, we successfully prepared two ethanol-based formulations *i.e.*, *Arka* and *Arista* from *Dhathaki Pushpa*, supporting the hypothesis that these extracts possess significant antimicrobial properties. The complex chemical profile of the aqueous extract indicates the need for further purification and analysis of the preparation to identify promising bioactive compounds in the source material. The findings of this study also emphasize the need for continued exploration of plant-derived extracts tagged with the application of modern science to uncover their full therapeutic potential.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS
During the preparation of this work the authors used chatgpt.com in order to check the quality of the language and grammar in the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

ABBREVIATIONS
Aqueous extract of *Dhataki Puspha* (*Dhathaki*-Aq), distilled extract of *Dhataki Puspha* (*Arka*; *Dhathaki*-A), and fermented preparation of *Dhataki Puspha* (*Arista*; *Dhathaki*-AA)

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