Comparative Phytochemical Analysis of Kanakasava, Kanakasava Distillate, and Kanakasava Nebulizer Solution using Gas Chromatography-mass Spectrometry (GC-MS)

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ABSTRACT

Introduction: Kanakasava is the classical formulation known to have beneficial effects on bronchial asthma. This study deals with the objective to compare the phytochemicals present in Kanakasava, Kanakasava distillate, and Kanakasava nebulizer solution using gas chromatography-mass spectrometry (GC-MS) and to determine if Kanakasava nebulizer solution can be useful in managing respiratory diseases. Methods: Kanakasava, Kanakasava distillate and Kanakasava nebulizer solution were prepared accordingly and standard GC-MS analysis was performed on the samples. Results: The GC-MS analysis indicated the presence of 6, 13 and 10 important phytochemicals in Kanakasava, Kanakasava distillate and Kanakasava nebulizer solution respectively. Many chemicals acting on the respiratory system were found in all three forms of Kanakasava and some of the chemicals were having similar actions. Conclusion: Despite the fact that the phytochemicals found in the three of them differed due to molecular fragmentation during the whole process, the efficacy of the novel Kanakasava nebulizer solution remained unaffected.

Keywords: Ayurveda, Kanakasava, Herbal nebulizer solution, GC-MS, Molecular fragmentation.

INTRODUCTION

Chronic respiratory disorders, which can affect both adults and children, are becoming more and more prevalent worldwide. According to WHO, an estimated 400 million individuals worldwide suffer from asthma and chronic obstructive pulmonary disease (COPD) alone. COPD prevalence is anticipated to increase over the next 40 years, with over 5.4 million deaths from COPD and related illnesses occurring annually by 2060. Despite a constant increase in the burden of chronic respiratory diseases, modern treatment including inhaled and oral corticosteroids, long and short-acting bronchodilators is not adequate and safe enough to manage respiratory diseases like asthma completely.
Many side effects like migraine, dry mouth, and tachycardia are seen with the prolonged use of modern anti-asthmatic therapy and studies have also shown that up to 80% of asthma-related deaths are caused by underlying bronchial inflammation due to common asthma inhalers. Considering the side effects that occur during modern therapy, there is a need to explore the traditional system of medicines that provides long-lasting and safe management for asthma.

Kanakasava is a classical Ayurvedic polyherbal formulation in which individual drug consists of some chemical constituents that are known to have anti-asthmatic, anti-allergy, anti-tussive, and bronchodilator actions. It makes the Kanakasava, a potent anti-asthmatic formulation when taken orally. It is a self-fermented formulation (Sandhana) and is useful in asthma (shwasa), cough (kasa), yakhsha (tuberculosis), kshatksheena (phthisis), chronic fever (jeerna jwara), and raktapitta (bleeding disorders). The immunostimulating activity of Kanakasava is proven through an in-vitro study which is seen due to its capacity to increase antibody production and splenocyte proliferation.

An animal study found Kanakasava to be effective against ovalbumin-induced bronchial asthma and airway inflammation in rats. The clinical efficacy of Kanakasava is also seen in the management of bronchial asthma as it provides symptomatic relief in the patients and statistically significant changes are seen in PEFR and FEV1 values. Therefore, it can be said that Kanakasava can be effective in the management of chronic respiratory diseases like asthma, COPD.

In cases of COPD or other diseases of the respiratory system, a medicine that can be directly administered into the lungs can show better and faster effects as compared to other routes. So, drug delivery through nebulizers or rota healers is highly effective. Hence, an effort was made to convert the Kanakasava into a nebulizer solution and to evaluate its efficacy in the management of respiratory diseases. The current study was carried out with the objective of comparing the phytochemicals present in Kanakasava, Kanakasava distillate, and Kanakasava nebulizer solution using gas chromatography-mass spectrometry (GC-MS) and to evaluate whether the Kanakasava nebulizer solution has the potential to manage chronic respiratory diseases or not.

**MATERIALS AND METHODS**

**Collection of Raw Plant Material**

All the crude drugs were collected and authenticated by an in-house expert botanist. The details of the ingredients are outlined in Table 1.

### Table 1: Kanakasava Composition

<table>
<thead>
<tr>
<th>S. No</th>
<th>Name of the Drug</th>
<th>Botanical Name</th>
<th>Part used</th>
<th>Chemical Constituent</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kanaka</td>
<td>Datura Stramonium Linn</td>
<td>Whole plant</td>
<td>Atropine, Scopolamine</td>
<td>Anticholinergic,[9] Bronchodilator[9]</td>
</tr>
<tr>
<td>6</td>
<td>Nagakesar</td>
<td>Mesua ferrea Linn</td>
<td>Stamen</td>
<td>Mesuaxanthone A, Mesuaxanthone B</td>
<td>Anti inflammatory,[15]</td>
</tr>
<tr>
<td>7</td>
<td>Shunthi</td>
<td>Zingiber officinalis Rosc</td>
<td>Rhizome</td>
<td>Zingerone, Gingerol</td>
<td>Anti inflammatory,[16]</td>
</tr>
<tr>
<td>9</td>
<td>Talispatra</td>
<td>Abies webbiana Lindl</td>
<td>Leaves</td>
<td>Saponins (Icosahydropicenic</td>
<td>Anti-inflammatory[18]</td>
</tr>
<tr>
<td>10</td>
<td>Dhataki</td>
<td>Woodfordia fruticosa (L.) Kurz.</td>
<td>Flowers</td>
<td>Octasal, diglucoside, and beta-sitosterol</td>
<td>Relieves cough[19]</td>
</tr>
</tbody>
</table>
Preparation of Kanakasava

Kanakasava was prepared according to the classical decoction method mentioned in Bhaishajya Ratnavali.20 All the crude drugs were initially cleaned, shade dried, powdered, and sieved. The powdered drugs were soaked in 25 litres of water and were kept undisturbed for 24 hours. Then, a porcelain jar was selected and Dhooopana was done using the Dhooopana Dravyas (Guggulu, Tulsi, Neem). Later the mixture was poured into the jar and at the end, Draksha (Vitis Vinifera) and flowers of Dhataki (Woodfordia fruticosa) were added and the container was sealed with a clay-smeared cloth. The container was kept undisturbed in a dark room for 38 days till the completion of fermentation. The following observations were used to confirm fermentation:

- Asava prepared possessed an alcoholic odour.
- No evidence of effervescence was seen.
- Burning candle test was positive.

After the confirmation of fermentation, the prepared formulation i.e., Kanakasava (4 litres) was filtered through a double-layered clean cotton cloth, stored in a glass flask and was subjected to distillation.

Preparation of Kanakasava Distillate

The distillate of Kanakasava was obtained through a simple distillation method.21 The following procedure was used to obtain the distillate. The distillation apparatus was set up which included a distillation flask, a condenser, and a receiving flask. The Kanakasava was poured into the distillation flask and was heated at 40 degree Celsius (°C) until it started boiling. As the Asava boils, the vapor rises and enters the condenser. The condenser cools the vapor, causing it to condense back into a liquid, which was collected in the receiving flask. The liquid collected in the receiving flask is the Kanakasava distillate (2 litres).

Preparation of Kanakasava nebulizer solution

The distillate thus obtained was diluted with distilled water in a ratio of 2:1, which was finalized after various trials as per the concentration suitable for nasal mucosa. The final solution obtained was the Kanakasava nebulizer solution which can be used directly for the nebulization.

Gas Chromatography-Mass Spectrometry Analysis

The GC-MS study was done by the protocols already available in the literature.22 The sample medicine was analysed using a GC-MS Perkin Elmer System, which included an autosampler and a gas chromatograph interfaced to a mass spectrometer (GCMS) apparatus, under the following conditions: The column used was Elite-5MS (30 meters*0.250mm*0.250um). Helium was used as a carrier gas at a constant flow rate of 1 mL/min, with an injection volume of 2 microlitres and an injector temperature of 260°C. The oven temperature was programmed to rise from 75°C (isothermal for 5 min) to 280°C at a rate of 10°C/min, then fall for 10 minutes at 280°C. The temperature of the EI source was 220°C. At a scan range of 20 to 610 amu, mass spectra were collected. The total running time for the GC was 45 minutes.

Identification of Compounds

For mass spectrum GC-MS interpretation, the National Institute of Standards and Technology (NIST) online database was used. The unknown component's mass spectrum was compared to the spectrum of known components listed in the NIST online database.22

RESULTS

The phytochemicals found in Kanakasava, Kanakasava Distillate, and Kanakasava nebulizer Solution were compared. They are listed below in Table 2, Table 3, and Table 4 respectively. [*Source: PubChem and Dr. Duke’s phytochemical and ethnobotanical database (online database)].
Table 2: Phytochemicals found in Kanakasava

<table>
<thead>
<tr>
<th>S. No</th>
<th>Name of Compound</th>
<th>Molecular Name</th>
<th>Molecular weight (g/mol)</th>
<th>Activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethyl 4-T-Butylbenzoate</td>
<td>C_{13}H_{18}O_{2}</td>
<td>206</td>
<td>Anti-microbial, Metabolite, Anti-tumor, Blood thinning</td>
</tr>
<tr>
<td>2</td>
<td>Pentanedioic acid</td>
<td>C_{19}H_{28}O_{4}</td>
<td>320</td>
<td>Metabolite, Acidifier, Anti-inflammatory, Anti-allergy</td>
</tr>
<tr>
<td>3</td>
<td>Phenol,2,5-BIS</td>
<td>C_{16}H_{16}O_{2}</td>
<td>206</td>
<td>Anti-oxidant, Anti-microbial, Anti-fungal, Anti-inflammatory,[23] Anti tuberculosis activity</td>
</tr>
<tr>
<td>4</td>
<td>Undecanoic acid</td>
<td>C_{22}H_{42}O_{2}</td>
<td>214</td>
<td>Antioxidant[24], inhibit production of Uric Acid, Anti fungal[25]</td>
</tr>
<tr>
<td>5</td>
<td>Decanoic acid</td>
<td>C_{12}H_{24}O_{2}</td>
<td>200</td>
<td>Anti-bacterial[26], Anti-fungal[26] Anti-inflammatory, Metabolite</td>
</tr>
<tr>
<td>6</td>
<td>Ethyl Tridecanoate</td>
<td>C_{15}H_{30}O_{2}</td>
<td>242</td>
<td>Anti-microbial[27], Anti-inflammatory[27]</td>
</tr>
</tbody>
</table>

Table 3: Phytochemicals found in Kanakasava distillate

<table>
<thead>
<tr>
<th>S. No</th>
<th>Name of Compound</th>
<th>Molecular Name</th>
<th>Molecular weight (g/mol)</th>
<th>Activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Malonic Acid, Dihydroxy-diisobutyl ester</td>
<td>C_{11}H_{20}O_{6}</td>
<td>248</td>
<td>Controlling acidity, Metabolite, Anti-bacterial action</td>
</tr>
<tr>
<td>2</td>
<td>Propane, 2-(1,1-Dimethylethyl Sulfonyl)-2-Methyl</td>
<td>C_{16}H_{14}O_{2}S</td>
<td>178</td>
<td>Anti-bacterial, Anti-fungal[29] Anti oxidant[29]</td>
</tr>
<tr>
<td>3</td>
<td>Oxalic acid, Bis(isobutyl) ester</td>
<td>C_{14}H_{18}O_{2}</td>
<td>202</td>
<td>Antioxidant, Antimicrobial</td>
</tr>
<tr>
<td>4</td>
<td>Triarachine</td>
<td>C_{16}H_{20}O_{2}</td>
<td>974</td>
<td>Anti-Microbial</td>
</tr>
<tr>
<td>5</td>
<td>Dodecyl Nonyl ether</td>
<td>C_{16}H_{20}O_{2}S</td>
<td>324</td>
<td>Anti-Microbial</td>
</tr>
<tr>
<td>6</td>
<td>Heptyl Hexadecyl ether</td>
<td>C_{12}H_{24}O_{2}</td>
<td>366</td>
<td>Anti-Microbial</td>
</tr>
<tr>
<td>7</td>
<td>Lauroyl Peroxide</td>
<td>C_{12}H_{24}O_{4}</td>
<td>398</td>
<td>Anti-helmintic[30], Anti-protozoal[30], Anti-viral[30], Anti-fungal[30]</td>
</tr>
<tr>
<td>8</td>
<td>Heptyl Octacosyl ether</td>
<td>C_{16}H_{32}O_{4}</td>
<td>508</td>
<td>Antimicrobial[31], Anti-bacterial, Anti oxidant[31]</td>
</tr>
<tr>
<td>9</td>
<td>Sulfurous acid, Cyclohexylmethyl Hexadecyl ester</td>
<td>C_{21}H_{32}O_{2}S</td>
<td>402</td>
<td>Anti-tumor[32], Antibacterial[33], Anti-Cancer[33]</td>
</tr>
<tr>
<td>10</td>
<td>Pimelic acid</td>
<td>C_{14}H_{20}O_{4}</td>
<td>426</td>
<td>E coli Metabolite</td>
</tr>
<tr>
<td>11</td>
<td>Trimethylpentyl Undecyl ester</td>
<td>C_{16}H_{30}O_{2}</td>
<td>426</td>
<td>Anti-microbial</td>
</tr>
<tr>
<td>12</td>
<td>Diethylmalonic acid</td>
<td>C_{16}H_{30}O_{2}</td>
<td>384</td>
<td>Synthesis of Anti-inflammatory agents, Flavoring agents, Controls acidity</td>
</tr>
<tr>
<td>13</td>
<td>Propanoic acid, 3,3'-thiobis-didodecyl ester</td>
<td>C_{23}H_{30}O_{4}S</td>
<td>514</td>
<td>Anti-microbial, Anti- leukotrienic agent[34] Anti- leukotrienic agent[34]</td>
</tr>
</tbody>
</table>

Table 4: Phytochemicals found in Kanakasava nebulizer solution

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of Compound</th>
<th>Molecular Name</th>
<th>Molecular Weight (g/mol)</th>
<th>Activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Salicyl Hydrazide</td>
<td>C_{7}H_{5}O_{2}N_{2}</td>
<td>152</td>
<td>Anti-microbial[35], Anti-bacterial, Anti-fungal</td>
</tr>
<tr>
<td>2</td>
<td>Phenyl Salicylate</td>
<td>C_{7}H_{6}O_{3}</td>
<td>214</td>
<td>Anti-bacteria[36], Anti-inflammatory</td>
</tr>
<tr>
<td>3</td>
<td>3-Eicosene</td>
<td>C_{21}H_{42}O_{2}</td>
<td>280</td>
<td>Anti-microbial[37], Antioxidant[38], Anti-inflammatory[38]</td>
</tr>
<tr>
<td>4</td>
<td>Cetene</td>
<td>C_{20}H_{40}O_{2}</td>
<td>224</td>
<td>Anti-bacterial[39], Anti-oxidant[39]</td>
</tr>
<tr>
<td>5</td>
<td>Trichloroacetic acid</td>
<td>C_{13}H_{18}O_{2}</td>
<td>386</td>
<td>Metabolite, used for treating acne, warts</td>
</tr>
<tr>
<td>6</td>
<td>Tetracosanol-1</td>
<td>C_{24}H_{50}O</td>
<td>354</td>
<td>Anti-Mutagenic[40], Antiseptic, Anti-inflammatory[41], Anti-bacterial[42]</td>
</tr>
<tr>
<td>7</td>
<td>Heptacosanol</td>
<td>C_{19}H_{36}O</td>
<td>396</td>
<td>(Flavouring agent, cholesterol Lowering, Antimicrobial and Antithrombotic)[42]</td>
</tr>
<tr>
<td>8</td>
<td>Cyclooctacosane</td>
<td>C_{22}H_{44}O_{2}</td>
<td>392</td>
<td>Anti-fungal, Anti-bacterial, Anti-inflammatory</td>
</tr>
<tr>
<td>9</td>
<td>Tricosene</td>
<td>C_{23}H_{46}O_{2}</td>
<td>322</td>
<td>Anti-bacterial[43]</td>
</tr>
<tr>
<td>10</td>
<td>Pentadecfluorooctanoic acid, Pentadecyl ester</td>
<td>C_{21}H_{31}O_{5}F_{15}</td>
<td>624</td>
<td>Anti-inflammatory[43]</td>
</tr>
</tbody>
</table>
Phytochemicals observed in all the three forms of Kanakasava, possess similar action on respiratory system. Details of the same are compiled in Tables 5 and 6.

Table 5: Chemical compounds with actions on the respiratory system

<table>
<thead>
<tr>
<th>S. No</th>
<th>Action</th>
<th>Kanakasava</th>
<th>Kanakasava distillate</th>
<th>Kanakasava nebulizer solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Anti-inflammatory</td>
<td>Pentanedioic acid, Phenol, 2,5-BIS</td>
<td>Malonic Acid, Dihydroxy-diisobutyl ester, Diethylylmalonic Acid</td>
<td>Phenyl Salicylate, 3-Eicosene, Tetracosanol-1, Cyclooctacosane, Pentadecafluoro-octanoic Acid, Pentadecyl Ester</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1,1-Dimethylethyl), Decanoic Acid, Ethyl Tridecanoate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Anti-bacterial</td>
<td>Decanoic Acid</td>
<td>Malonic Acid, Dihydroxy-diisobutyl ester, Propane, 2-(1,1-Dimethylethyl Sulfonyl) -2-Methyl, Heptyl Octacosyl Ether, Sulfurous acid, Cyclohexylmethyl Hexadecyl Ester</td>
<td>Salicyl Hydrazide, Phenyl Salicylate, Tetracosanol-1, Cyclooctacosane, Tricosene</td>
</tr>
<tr>
<td>3</td>
<td>Anti-fungal</td>
<td>Phenol, 2,5-BIS (1,1-Dimethylethyl), Undecanoic Acid, Decanoic Acid</td>
<td>Propane, 2-(1,1-Dimethylethyl Sulfonyl)-2-Methyl, Lauroyl Peroxide,</td>
<td>Salicyl Hydrazide, Cyclooctacosane</td>
</tr>
<tr>
<td>4</td>
<td>Anti-microbial</td>
<td>Ethyl 4-T-Butylbenzoate, Phenol, 2,5-BIS (1,1-Dimethylethyl), Ethyl Tridecanoate</td>
<td>Oxalic Acid, Bis(isobutyl) Ester, Triarachine, Dodecyl Nonyl Ether, Heptyl Hexadecyl Ether, Heptyl Octacosyl Ether, Trime thylpentyl Undecyl Ester, Propanoic acid, 3,3’-thiobis-didodecyl Ester</td>
<td>Salicyl Hydrazide, 3-Eicosene, Cetene, Heptacosanol</td>
</tr>
<tr>
<td>5</td>
<td>Anti-oxidant</td>
<td>Phenol, 2,5-BIS (1,1-Dimethylethyl), Undecanoic Acid</td>
<td>Propane, 2-(1,1-Dimethylethyl Sulfonyl)-2-Methyl, Oxalic Acid, Bis(isobutyl) Ester, Heptyl Octacosyl Ether</td>
<td>3-Eicosene, Cetene</td>
</tr>
<tr>
<td>6</td>
<td>Anti-tuberculotic activity</td>
<td>Phenol, 2,5-BIS (1,1-Dimethylethyl)</td>
<td>Propanoic acid, 3,3’-thiobis-didodecyl Ester</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Anti-leukotrienic agent</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6: Chemical compounds having similar actions

<table>
<thead>
<tr>
<th>Chemical compounds</th>
<th>Sample</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decanoic acid[44]</td>
<td>Kanakasava</td>
<td>Anti-bacterial action against Staphylococcus aureus</td>
</tr>
<tr>
<td>Sulfurous acid, Cyclohexylmethyl</td>
<td>Kanakasava Distillate</td>
<td></td>
</tr>
<tr>
<td>Hexadecyl Ester[45]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-Eicosene[46], Phenyl Salicylate[47]</td>
<td>Kanakasava Nebulizer Solution</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

This study shows that although molecule fragmentation has increased from fermented form (Asava) to nebulizer form, many of the molecular fragments are dissociated into a composite chemical. It is seen that through the fragmentation of molecules, new chemicals have been identified which has been the case with Kanakasava nebulizer. In Kanakasava, a total of 6 identifiable compounds were found, in Kanakasava distillate, 13 identifiable compounds were found and in Kanakasava nebulizer solution, 10 identifiable compounds were found. Factors like high temperature, type of water (distilled, deionized, or tap water) used for the dilution, atmospheric conditions, or variation in the pH of the solutions after the distillation or dilution process can affect the fragmentation of molecules in any experiment. However, in this study, high temperature, atmospheric conditions or variations in the pH of the solutions after the distillation or dilution
process might have contributed to the fragmentation of molecules.

Even though there were a number of steps involved in the process and it is possible that some sensitive organic molecules might have resulted in fragmentation during different steps under GC-MS and our results indicate that fragmentation at different steps does not affect the effectiveness of the novel nebulizer solution reported in this work. The molecular fragments obtained are of the finest forms of organic compounds and they show similar actions like antibacterial action, anti-inflammatory action which were found in all three forms of Kanakasava (as shown in Table 4.) For example, all forms of Kanakasava, including Kanakasava, Kanakasava distillate, and Kanakasava nebulizer solution, have shown anti-bacterial activity against Staphylococcus aureus which exerts a pathogenetic role in many chronic airway illnesses, such as COPD, asthma, pneumonia, etc.48 (Table 5).

This indicates that both the Kanakasava and the Kanakasava nebulizer solution would aid in preventing and treating chronic respiratory infections. With the help of the finest fragments present in the Kanakasava nebulizer solution, respiratory disorders can be treated more successfully by ensuring the targeted delivery of the necessary medication.

Limitations and Future Scope of the Study

To identify more phytochemical elements in detail in all three forms, various analytical tests, such as LCMS and HPTLC, can be conducted. The results of all the analytical tests can then be compared. Kanakasava nebulizer solution can go through all four stages of clinical trials following a thorough examination of the drug so that it can be utilized in the future to treat chronic respiratory disorders like COPD and asthma.

CONCLUSION

When a fundamental formulation is transformed into various new forms, the phytochemicals that result may vary due to various preparation methods and proper molecular fragmentation, but the new forms will still exhibit the same action as the fundamental formulation, only the chemical composition of molecules may change. Additionally, converting the well-known asthma medication Kanakasava into a nebulizer solution can aid in the treatment of respiratory illnesses.

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Conflict of interest

There is no conflict of interest, according to the authors.

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