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Comparative Phtyochemical 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[1]. 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[2]. 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[3]. 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[4].

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)*, yakshma (tuberculosis), *kshatksheena* (~phthisis), chronic fever *(jeerna jwara)*, and *raktapitta (~bleeding disorders)*[5]. 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[6]. An animal study found *Kanakasava* to be effective against ovalbumin-induced bronchial asthma and airway inflammation in rats[7]. 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[8]. 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

S. No	Name of the Drug	Botanical Name	Part used	Chemical Constituent	Action
1	Kanaka	*Datura Stramonium* Linn	Whole plant	Atropine, Scopolamine	Anticholinergic,[9] Bronchodilator[9]
2	Vasa	*Adhatoda vasica* Nees	Whole plant	Vasicine (Bromhexine and Ambroxol), Vasicinone, Vascinol	Anti-asthmatic,[10] Bronchodilator,[11] Anti-allergy,[11] Anti tubercular[11]
3	Madhuka	*Glycyrrhiza glabra* Linn.	Root	Glycyrrhizin, Liquiritin, Liquiritigenin	Anti-tussive,[12] Expectorant[12]
4	Pippali	*Piper longum* Linn	Fruits	Piperine, Piplartine	Anti asthmatic,[13] Bronchodilator,[13] Anti inflammatory[13]
5	Kantakari	*Solanum xanthocarpum* Scrad & Wendl	Whole plant	Solanacarpine, Solamargine, Solasodine	Anti inflammatory,[14] Anti-asthmatic, [14] Anti-tussive
6	Nagakesar	*Mesua ferrea* Linn	Stamen	Mesuaxanthone A, Mesuaxanthone B	Anti inflammatory[15]
7	Shunthi	*Zingiber officinalis Rosc*	Rhizome	Zingerone, Gingerol	Anti inflammatory[16]
8	Bharangi	*Clerodendrum serratum* (Linn.)	Root	Catechin, Luteolin	Anti asthmatic,[17] Anti histaminic,[17] Anti-allergy[17]
9	Talispatra	*Abies webbiana Lindl*	Leaves	Saponins (Icosahydropicenic	Anti-inflammatory[18]
10	Dhataki	*Woodfordia fruticosa* (L.) Kurz.	Flowers	Octasonal, diglucoside, and beta-sitosterol	Relieves cough[19]

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 *Dhoopana* was done using the *Dhoopana 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 (^{0}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*

S. No	Name of Compound	Molecular Name	Molecular weight (g/mol)	Activity*
1	Ethyl 4-T-Butylbenzoate	$C_{13}H_{18}O_2$	206	Anti-microbial, Metabolite, Anti-tumor, Blood thinning
2	Pentanedioic acid	$C_{19}H_{28}O_4$	320	Metabolite, Acidifier, Anti-inflammatory, Anti-allergy
3	Phenol,2,5-BIS (1,1-Dimethylethyl)	$C_{14}H_{22}O$	206	Anti-oxidant, Anti-microbial, Anti-fungal, Anti-inflammatory,[23] Anti tuberculotic activity
4	Undecanoic acid	$C_{13}H_{26}O_2$	214	Antioxidant[24], inhibit production of Uric Acid, Anti fungal[25]
5	Decanoic acid	$C_{12}H_{24}O_2$	200	Anti-bacterial[26], Anti-fungal,[26] Anti-inflammatory, Metabolite
6	Ethyl Tridecanoate	$C_{15}H_{30}O_2$	242	Anti-microbial[27], Anti-inflammatory[27]

Table 3: Phytochemicals found in *Kanakasava* distillate

S. No	Name of Compound	Molecular Name	Molecular Weight(g/mol)	Activity*
1	Malonic Acid, Dihydroxy-diisobutyl ester	$C_{11}H_{20}O_6$	248	Controlling acidity, Metabolite, Anti-inflammatory[28], Anti-bacterial action
2	Propane, 2-(1,1-Dimethylethyl Sulfonyl)-2-Methyl	$C_8H_{18}O_2S$	178	Anti-bacterial, Anti-fungal,[29] Anti oxidant[29]
3	Oxalic acid, Bis(isobutyl) ester	$C_{10}H_{18}O_4$	202	Antioxidant, Antimicrobial
4	Triarachine	$C_{63}H_{122}O_6$	974	Anti-Microbial
5	Dodecyl Nonyl ether	$C_{19}H_{32}0_2S$	324	Anti-Microbial
6	Heptyl Hexadecyl ether	$C_{26}H_{54}$	366	Anti-Microbial
7	Lauroyl Peroxide	$C_{24}H_{46}O_4$	398	Anti -helminthic[30], Anti-protozoal[30], Anti-viral[30], Anti-fungal[30]
8	Heptyl Octacosyl ether	$C_{35}H_{72}0$	508	Antimicrobial[31], Anti-bacterial, Antioxidant[31]
9	Sulfurous acid, Cyclohexylmethyl Hexadecyl ester	$C_{23}H_{46}O_3S$	402	Anti-tumor[32], Antibacterial[33], Anti-Cancer[33]
10	Pimelic acid	$C_{26}H_{50}O_4$	426	*E coli* Metabolite
11	Trimethylpentyl Undecyl ester	$C_{26}H_{50}O_4$	426	Anti-microbial
12	Diethylmalonic acid	$C_{23}H_{44}O_4$	384	Synthesis of Anti-inflammatory agents, Flavoring agents, Controls acidity
13	Propanoic acid, 3,3'-thiobis-didodecyl ester	$C_{30}H_{580}O_4S$	514	Anti-microbial, Anti-leukotrienic agent[34]

Table 4: Phytochemicals found in Kanakasava nebulizer solution

S.No	Name of Compound	Molecular Name	Molecular Weight (g/mol)	Activity*
1	Salicyl Hydrazide	$C_7H_8O_2N_2$	152	Anti-microbial[35], Anti-bacterial, Anti-fungal
2	Phenyl Salicylate	$C_{13}H_{10}O_3$	214	Anti-bacterial[36], Anti-inflammatory
3	3-Eicosene	$C_{20}H_{40}$	280	Anti-microbial[37], Antioxidant[38], Anti-inflammatory[38]
4	Cetene	$C_{16}H_{32}$	224	Anti-microbial[39], Anti-oxidant[39]
5	Trichloroacetic acid	$C_{18}H_{33}O_2C_{13}$	386	Metabolite, used for treating acne, warts
6	Tetracosanol-1	$C_{24}H_{50}O$	354	Anti-Mutagenic[40], Antiseptic, Anti-inflammatory[41], Anti -bacterial[42]
7	Heptacosanol	$C_{27}H_{56}O$	396	(Flavouring agent, cholesterol Lowering, Antimicrobial and Antithrombotic)[42]
8	Cyclooctacosane	$C_{28}H_{56}$	392	Anti-fungal, Anti-bacterial, Anti-inflammatory
9	Tricosene	$C_{23}H_{46}$	322	Anti-bacterial[43]
10	Pentadecafluorooctanoic acid, Pentadecyl ester	$C_{23}H_{31}O_2F_{15}$	624	Anti-inflammatory[43]

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

S. No	Action	*Kanakasava*	*Kanakasava* distillate	*Kanakasava* nebulizer solution
1	Anti-inflammatory	Pentanedioic acid, Phenol,2,5-BIS (1,1-Dimethylethyl), Decanoic Acid, Ethyl Tridecanoate	Malonic Acid, Dihydroxy-diisobutylester, Diethylmalonic Acid	Phenyl Salicylate, 3-Eicosene, Tetracosanol-1, Cyclooctacosane, Pentadecafluorooctanoic Acid, Pentadecyl Ester
2	Anti-bacterial	Decanoic Acid	Malonic Acid, Dihydroxy-diisobutyl ester, Propane, 2-(1,1-Dimethylethyl Sulfonyl) -2-Methyl, Heptyl Octacosyl Ether, Sulfurous acid, Cyclohexylmethyl Hexadecyl Ester	Salicyl Hydrazide, Phenyl Salicylate, Tetracosanol-1, Cyclooctacosane, Tricosene
3	Anti-fungal	Phenol,2,5-BIS (1,1-Dimethylethyl), Undecanoic Acid, Decanoic Acid	Propane, 2-(1,1-Dimethylethyl Sulfonyl)-2-Methyl, Lauroyl Peroxide,	Salicyl Hydrazide, Cyclooctacosane
4	Anti-microbial	Ethyl 4-T-Butylbenzoate, Phenol,2,5-BIS (1,1-Dimethylethyl), Ethyl Tridecanoate	Oxalic Acid, Bis(isobutyl) Ester, Triarachine, Dodecyl Nonyl Ether, Heptyl Hexadecyl Ether, Heptyl Octacosyl Ether, Trimethylpentyl Undecyl Ester, Propanoic acid, 3,3'-thiobis-didodecyl Ester	Salicyl Hydrazide, 3-Eicosene, Cetene, Heptacosanol
5	Anti-oxidant	Phenol,2,5-BIS (1,1-Dimethylethyl), Undecanoic Acid	Propane, 2-(1,1-Dimethylethyl Sulfonyl)-2-Methyl, Oxalic Acid, Bis(isobutyl) Ester, Heptyl Octacosyl Ether	3-Eicosene, Cetene
6	Anti-tuberculotic activity	Phenol,2,5-BIS (1,1-Dimethylethyl)		
7	Anti-leukotrienic agent		Propanoic acid, 3,3'-thiobis -didodecyl Ester	

Table 6: Chemical compounds having similar actions

Chemical compounds	Sample	Action
Decanoic acid[44]	Kanakasava	Anti-bacterial action against *Staphylococcus aureus*
Sulfurous acid, Cyclohexylmethyl Hexadecyl Ester[45]	Kanakasava Distillate	
3-Eicosene[46], Phenyl Salicylate[47]	Kanakasava Nebulizer Solution	

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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