ABSTRACT

This research is centered on the formulation known as “Sarpdansh Amrita” developed by a Vaidya in the Jind district of Haryana, India. This formulation is employed by the local community as a first-aid remedy for snake bites and aphid stings. The study aims at physicochemical and phytochemical evaluation in methanolic extract of formulation. A total of 16 biochemical compounds were identified using GC-MS. The compounds identified in the formulation were compared with the mass spectra of the National Institute of Standards and Technology (NIST). The major identified phytochemicals include Lupeol; Lup-20(29)-en-3-one; Cis-13-Octadecenoic acid, methyl ester; Hexadecanoic acid, methyl ester; Bergamotol, Z-â-trans-; 9,12-Octadecadienoic acid (Z,Z)-, methyl ester; Undecanoic acid; Lanceol, cis, etc. The formulation holds promising pharmacological potential due to its abundant content of bioactive phytochemicals. The identified molecules can be further isolated, purified, and studied for pharmacological properties concerning their effect in case of snake bites.

Key words: Physico-chemical, Phytochemicals, GC-MS, Sarpdansh Amrita, Snake bites.

Introduction

Snake bite incidents pose a significant public health challenge globally, contributing to an annual death toll that ranges astonishingly from 25,000 to 125,000 fatalities and leaving countless individuals with enduring disabilities (Gutierrez et al., 2015). In acknowledgment of their impact, the World Health Organization added snake envenomation to the list of Neglected Tropical Diseases (NTDs) in 2009. Particularly affected are populations in rural tropics and subtropics, where limited access to healthcare is prime reason of mortality and morbidity (Harrison et al., 2009).

When snake bites, venom is injected into victims via fangs originating from venom glands of snake (Kardong et al., 1993). The snake venom contains pharmacologically active proteins and polypeptide complexes designed to immobilize prey. The consequences of snake envenomation can range from immediate effects to severe systemic outcomes, encompassing inflammation, oxidative stress, swelling, lipolysis, muscle necrosis, skin tissue damage, kidney and cardiovascular function disturbances (Kang et al., 2011).

The primary approach to treating snake venom involves the use of antivenom serum therapy. However, this method is encumbered by high costs, limited availability, and a significant risk of immunological reactions, whether immediate or delayed (Ryan et al., 2016). Given these challenges, the exploration of alternative snakebite treatments becomes imperative. Medicinal plants and traditional remedies hold promise for yielding compounds with antiophidic properties. Contemporary toxicologists are increasingly turning to traditional folk remedies due to their affordability, stability, and potential efficacy against venom toxins (Butt et al.,...
The assessment of local formulations plays a pivotal role in evaluating their effectiveness, potential toxicity, and quality control. To explore alternative medicines local practitioners known as “Vaids” were engaged as knowledgeable sources to collect relevant insights and a formulation was selected. The selection of a specific formulation was guided by its significance and feedback from individuals who had experienced positive outcomes from its use. In the Jind District of Haryana, a polyherbal preparation named “Sardansh Amrita” has gained attention among locals as an initial and preventive measure against snakebites, especially when immediate medical aid is lacking. This formulation also reported efficacy against aphid stings. The ongoing study encompasses physico-chemical and phytochemical assessments, coupled with GC-MS analysis, to scientifically evaluate the chemical composition of Sarpdansh Amrita (SA) as the formulation is not thoroughly accessed for the above properties. Transition from traditional to contemporary drugs can be accelerated through scientific examination of formulation.

**Materials and Methods**

**Collection of study material**

Ingredients of formulation are Sapindus mukorossi (fruit), family Sapindaceae, Safed Katha (Khadira), Neela Thotha (Copper sulphate) and Kalmi Shora (Potassium nitrate), cow ghee. *Sapindus mukorossi* (fruit) was purchased from a medical herb store and was authenticated from NISCAIR (National Institute for Science Communication and Policy Research), Pusa Gate, New Delhi with authentication no. – NIScPR/RJMD/Consult/2023/4485-86-7 12/6/2023. Safed Katha, Neela Thotha, and Kalmi shora were purchased from INDIAN JADIBOOTI registered under FSSAI license no.13319011000193.

**Preparation of in-house formulation**

An in-house formulation was prepared under the guidance of a local practitioner. Final product was prepared in the form of tablets. The sample was stored in an airtight jar at room temperature for further analysis.

**Preparation of extract**

For analysis, a methanolic extract was prepared. The formulation was mixed with methanol in an Erlenmeyer flask, sealed with aluminium foil, and left undisturbed for 24 hours. Afterward, it was agitated in a shaker for 24 hours and then filtered using Whatman filter paper no. 1. The filtered solution was subsequently evaporated to yield dried extract, which was used to create a stock solution in methanol.

**Organoleptic evaluation**

Organoleptic parameters of formulation such as colour, odour, taste and texture were evaluated by sense organs.

**Physicochemical analysis**

The parameters like pH, loss on drying (moisture content at 105°C), water-soluble extractives, alcohol-soluble extractives, total ash, acid-insoluble ash and water-soluble ash of the formulation were evaluated. The procedure was followed as per Standard Operating Procedures [Ayurvedic Pharmacopoeia of India (API), 2001].

**Qualitative phytochemicals screening**

Preliminary phytochemical screening of methanolic extracts of the formulation was done used to identify phytoconstituents as per standard guidelines (API, 2001).

**Quantitative phytochemical screening**

**Total phenolic content**

The phenolic content of the extract was determined using the Folini-Ciocalteu reagent (FCR) method (Cicco et al., 2009). To plot the standard curve, gallic acid was taken at different concentrations of (25-250µg/ml). The absorbance was read at 750nm. Results were expressed in µg GAE/mg. Triplicates of the assay were done.

**Total Flavonoid content**

The total flavonoid content (TFC) was estimated using aluminium chloride colorimetric assay (Ebrahimzadeh et al., 2008). To plot the standard curve quercetin was taken at different concentrations (0.6-10 µg/ml). The absorbance was read at 415nm. Results were expressed as µg QE/mg of plant extract. Triplicates of the assay were done.

**Total Terpenoid content**

The total terpenoid content (TTC) was estimated using the method described by Ghorai et al. (2012). To plot the standard curve linalool was taken at different concentrations (78-2500 µg/ml). The absorbance was read at 538nm. Results were expressed as µg Linalool/mg of plant extract. Triplicates of the assay were done.

**Total Tannin content**

Total tannin content was estimated using the method described by Oikeh et al. (2020). To plot the standard curve tannic acid was taken at different concentrations (0.1-10 µg/ml). The absorbance was read at 725nm. Results were expressed as µg Tannic acid /mg of plant extract. Triplicates of the assay were done.
Analysis of methanolic extract for secondary metabolites by Gas Chromatography-Mass Spectrometry

GC-MS analysis of methanolic extract of the formulation was done at SAIF-CIL, Panjab University, Chandigarh using the Thermo Scientific 1300 GC coupled with thermos TSQ8000 triple quadrupole MS was used. The capillary column (30*0.25µmID*0.25µmdf) with 5% diphenyl and 95% dimethyl polyxylane was used. The total run time of GC was 20.11 minutes and the injection volume was 1µl. Helium was used as carrier gas having a flow rate of 1ml/minute. The injector temperature, ion source temperature, oven temperature were maintained at 250°C, 250°C and 230°C, respectively. The mass spectrum was taken within the range of 50-600 at 70eV ionization voltage. Data interpretation was done using the National Institute Standard and Technology (NIST) and the identity of phytoconstituents was ascertained.

Results and Discussion

Vaidya’s choice of ingredients stands validated, as a wealth of literature supports the selection of these components. These ingredients have found application across various ailments, substantiating their utility in disease management.

Sapindus mukorossi is a member of the Sapindaceae family and its pericarp of fruit is used in the formulation. It is traditionally used as an expectorant, natural detergent and abortifacient. Fruit is reported to have anti-inflammatory, hepatoprotective, antiplatelet aggregation activities (Ibrahim et al., 2008 and de Queiroz et al., 2017). Safed katha, also identified as Khadira, is sourced from the heartwood of Acacia catechu. In the treatment of snakebites, a formulation containing katha is orally administered to patients. It exhibits properties such as antioxidant, anti-inflammatory, antibacterial and antifungal. It helps in protecting the liver and wound healing and treating pain (Stohs et al., 2015). Neela thotha known as copper sulphate is a mineral origin drug. Despite its inherent toxicity, after purification it has utility within the framework of the Unani system of medicine. It is used in leprosy, bronchitis, chronic leucorrhoea, chronic gonorrhoea, epilepsy, tremor and piles. Once purified, it becomes a valuable component in the treatment of poisoning and induce emesis (Ahmad et al., 2021). Kalmi shora, or potassium nitrate, presents as a white crystalline salt. This compound showcases diuretic, anti-inflammatory, expectorant, vasodilator properties and pain relieving properties (Kalam et al., 2023).

The results of the organoleptic evaluation are depicted in Table 1. The organoleptic observations indicate that the prepared Sarpdansh Amrita is in the form of a dark brown tablet with a characteristic aroma and flavour.

The mean value of pH, moisture content at 105°C, ash values (total ash, water-soluble ash and acid-insoluble ash), and extractive values (alcohol-soluble and water-soluble) are depicted in Table 2. Qualitative phytochemical screening for carbohydrates, phenols, flavonoids, saponins, tannins, steroids, glycosides, amino acids, terpenoids, and tannins was done and showed positive results for carbohydrates, phenols, flavonoids, saponins, glycosides, terpenoids and tannins. The results of quantitative phytochemical screening of the methanolic extract of the formulation are depicted in Table 3. In both qualitative and quantitative phytochemical analyses, the formulation demonstrates a scant presence of phenols (30.65 ± 5.42 µg GAE/mg of extract) and flavonoids (191.70 ± 5.03 µg QE acid/mg of extract), while showing a significant abundance of terpenoids (582.06 ± 83.36 µg LE /mg of extract), and tannins (390 ± 8 µg TAE /mg of extract).

The GC-MS analysis of the methanolic extract of the formulation was done at SAIF-CIL, Panjab University, Chandigarh using the Thermo Scientific 1300 GC coupled with thermos TSQ8000 triple quadrupole MS was used. The capillary column (30*0.25µmID*0.25µmdf) with 5% diphenyl and 95% dimethyl polyxylane was used. The total run time of GC was 20.11 minutes and the injection volume was 1µl. Helium was used as carrier gas having a flow rate of 1ml/minute. The injector temperature, ion source temperature, oven temperature were maintained at 250°C, 250°C and 230°C, respectively. The mass spectrum was taken within the range of 50-600 at 70eV ionization voltage. Data interpretation was done using the National Institute Standard and Technology (NIST) and the identity of phytoconstituents was ascertained.

### Table 1: Observations of organoleptic analysis of SA.

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Properties</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Description</td>
<td>Solid tablets</td>
</tr>
<tr>
<td>2.</td>
<td>Color</td>
<td>Dark brown</td>
</tr>
<tr>
<td>3.</td>
<td>Odor</td>
<td>Characteristic</td>
</tr>
<tr>
<td>4.</td>
<td>Taste</td>
<td>Characteristic</td>
</tr>
<tr>
<td>5.</td>
<td>Texture</td>
<td>Smooth</td>
</tr>
</tbody>
</table>

### Table 2: Observations of physicochemical analysis of SA.

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Properties</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>pH</td>
<td>2.47</td>
</tr>
<tr>
<td>2.</td>
<td>Moisture content</td>
<td>5.07±0.60%</td>
</tr>
<tr>
<td>3.</td>
<td>Total Ash</td>
<td>34.12±2.13%</td>
</tr>
<tr>
<td>4.</td>
<td>Acid Insoluble Ash</td>
<td>14.55±1.34%</td>
</tr>
<tr>
<td>5.</td>
<td>Water soluble ash</td>
<td>27.92±1.13%</td>
</tr>
<tr>
<td>6.</td>
<td>Alcohol soluble extractive</td>
<td>19.46±0.94 %</td>
</tr>
<tr>
<td>7.</td>
<td>Water soluble extractive</td>
<td>24±1.6%</td>
</tr>
</tbody>
</table>

### Table 3: Quantitative phytochemical analysis of methanolic extract of SA.

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Phytochemical analysis</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Phenolic content (µgGAE/mg of extract)</td>
<td>30.65±5.42</td>
</tr>
<tr>
<td>2.</td>
<td>Flavanoid content (µgQE/mg of extract)</td>
<td>191.70±5.03</td>
</tr>
<tr>
<td>3.</td>
<td>Terpenoid content (µgLE/mg of extract)</td>
<td>582.06±83.36</td>
</tr>
<tr>
<td>4.</td>
<td>Tannin content (µgTAE/mg of extract)</td>
<td>390±8</td>
</tr>
</tbody>
</table>

The results of the organoleptic evaluation are depicted in Table 1. The organoleptic observations indicate that
the formulation shows the presence of 16 phytochemical compounds in the composition. The active principles with their retention (RT), molecular formula (MF), molecular weight (MW) and concentration (peak area percentage) are depicted in Table 4. The mass spectra of identified phytochemicals are depicted in Figure 1.A comprehensive list of 16 bioactive compounds was identified through GC-MS assessment of the methanolic extract viz. Lupeol (19.20%); Lup-20(29)-en-3-one (15.79%); Cis-13-Octadecenoic acid, methyl ester (15.45%); Hexadecanoic acid, methyl ester (10.46%); Bergamotol, Z-à-trans (7.46%); Sitosterol (5.99%); 3-Acetoxy-3-hydroxypropionic acid, methyl ester (5.13%); Methyl stearate (3.82%); Methyl tetradecanoate (2.90%); 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (1.96%); Undecanoic acid (1.81%); Lanceol, cis (1.42%); 1-Isopropenyl-3-propenylcyclopentanone (1.16%); 2,6,10-Dodecatrien-1-ol, 12-acetoxy 2,6,10-trimeth, (E,E,E)- (1.26%); Diepicedrene-1-oxide (0.76%) and 1-Ethyl-4,4-dimethyl-cyclohex-2-en-1-ol (0.90%). In silico data of molecular docking properties to study absorption-distribution-metabolism-excretion-toxicity for these compounds was collected from the database of Indian Medicinal Plants, Phytochemistry and Therapeutics.

Diepicedrene-1-oxide is a sesquiterpenoid and an oxygenated component may function as an antioxidant (Assaeed et al., 2020). It has high blood brain permeation and gastrointestinal absorption ability. It is potentially as good pharmacokinetic drug as its successfully passes the Lipinski’s rule of 5 and Ghose rule. 10-Methyl undecanoic acid is non-toxic to mammalian cells and possesses antifungal properties (Rossi et al., 2021). It is component of RS-1 analog of teicoplanin glycopeptide antibiotic clinically effective against gram positive bacterial infections (Campoli-Richards, 1990). Cis-Lanceol is a sesquiterpenoid demonstrates effectiveness against pneumolysin toxin, which modulates inflammatory responses (Hirst et al., 2004). It has good blood brain barrier permeation and gastrointestinal absorption abilities having bioavailability score of 0.55. It passes the Lipinski’s rule of 5 and Ghose rule. n-Hexadecanoic acid commonly called as palmitic acid is saturated fatty acid. It is reported to have antioxidant, anti-inflammatory, hypocholesterolemic, antitumor, immunostimulant, haemolytic, 5-α-reductase inhibitor, lipoxigenase inhibitor properties (Ganesan et al., 2022). Structural and kinetic studies demonstrate that n-Hexadecanoic acid is an inhibitor of phospholipase A2. PLA2s are prevalent in reptile and arthropod venoms (Aparna et al., 2012). Thus, n-Hexadecanoic acid may serve as a superior candidate for anti-phospholipase A2 drugs showing promise as antidotes against a wide range of snake venoms and other animal toxins (Xiao et al., 2017). It successfully passes the Lipinski’s rule of 5 and Ghose rule. It has high blood brain barrier permeation ability and good gastrointestinal absorption ability with high bioavailability score of 0.85.9,12-Octadecadienoic acid (Z, Z) commonly called as linoleic acid is omega-6-unsaturated acid is an anti-inflammatory activity (Pinto et al., 2017 and Mori et al., 2013). It is a component of cell membrane and an Arachidonic acid (ARA) precursor (Tallima et al., 2018). It showcases different properties such as hypocholesterolemic, antihistaminic, 5-α-reductase inhibitor, antiarthritic and antioxidant properties. It passes Lipinski’s rule of 5 and Ghose rule having the bioavailability score of 0.85. Cis-13-octadecenoic acid, also known as petroselinic acid, is a mono-unsaturated fatty
acid influences the lipolysis. It has anti-inflammatory, anticancer, hypocholesterolemic properties (Delbeke et al., 2008). Another contributor, lupeol is a pentacyclic triterpenoid, is non-toxic to cells and tissues. Its potent the reduction of prostaglandin E2 (PGE2) production leads to anti-inflammatory activity. It passes Lipinski’s rule of 5, failed in the Ghose rule having bioavailability score 0.55. Studies show that it can inhibit reactive oxygen species production and restore anti-oxidant levels. Lupeol having the greatest peak area percentage is known to reduce the generation of proinflammatory cytokines and exhibit high wound-healing potential (Fernandez et al., 2001).

Phytochemical identified in this study shows that this formulation is a potential candidate for investigation as remedy of snake bite. Compounds identified are notable for having wound healing properties, anti-inflammatory activities, PLA2 inhibition capabilities and others. On basis of data made available by this study researchers can conceptualize the mechanisms and action of phytochemicals in the body. This can be effective in study of identified phytochemicals to get insights of interactions that are possible for effectiveness of traditional drugs.

### Conclusion

The components utilized in this study are believed to play a significant role in addressing toxicity resulting from snake bites. The outcomes of this research could contribute to a more reasoned evaluation and assessment of the versatile therapeutic applications of the formulated mixture. This study has broadened the possibilities for developing innovative drugs by phytochemical drugs derived from this composition. These drugs have the potential to serve as enhanced therapeutic agents, promoting awareness of the importance of exploring locally utilized remedies for addressing envenomation cases.

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