

“Solvent-Dependent Phytochemical Profiling of *Saraca asoca* Flowers: A Comparative GC-MS Study”

Ankita Yadav^{1#}, Karthika Murukan^{1#}, Poonam Pal¹, S. Krishnamoorthi², Anurag Mishra¹, Komal Jayram Wadekar¹, Sanjeev Kumar^{1*}

¹Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi – 221005, Uttar Pradesh, India

²Department of Chemistry, Institute of Science, Banaras Hindu University, Varanasi – 221005, Uttar Pradesh, India

^{1#}Equally contributed first authorship

*Correspondence Author: Dr. Sanjeev Kumar

*Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005, Uttar Pradesh, India Email ID: kumarsanjeevdg@bhu.ac.in

Abstract:

Saraca asoca (Roxb.) Wild., a revered medicinal plant in Ayurvedic medicine, has gained attention for its therapeutic properties. However, a comprehensive comparison of its phytochemical profile across different solvents remains unexplored. This study introduces a novel approach by employing Gas Chromatography-Mass Spectrometry (GC-MS) to analyze *S. asoca* flower extracts using a range of solvents- alcoholic, aqueous, and hydroalcoholic solutions.

The key innovation of this work lies in its comparative analysis of solvent-dependent variations in the extraction of bioactive compounds, including glycosides, anthocyanins, and flavonoids. By examining how solvent polarity and affinity influence phytochemical yield, this study reveals unexpected differences in compound solubility and bioactivity, offering deeper insights into the plant's pharmacological potential.

Unlike previous research focused on single-solvent extractions, our study unveils solvent-specific profiles, providing a clearer understanding of the plant's phytochemical diversity. These findings offer new avenues for optimizing *S. asoca*'s therapeutic applications in drug discovery, nutraceuticals, and pharmacognosy. Furthermore, the data provides a foundation for more targeted research into the plant's bioactive components, enhancing its potential for modern medicinal use.

Keywords: Bioactive compounds, GC-MS analysis, Flower, *Saraca asoca*, Antioxidant, Antimicrobial activity.

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Introduction:

"*Saraca asoca*", commonly known as *Asoka* or *Ashoka*, is a revered plant in Ayurvedic medicine, with a rich historical use in treating a range of ailments. The name *Asoka*, derived from Sanskrit, translates to "without sorrow" or "that does not cause grief," reflecting its cultural and medicinal importance.¹⁻³ Found across the Himalayas and in the southern regions of India, this medium-sized tree in the Fabaceae family is celebrated for its fragrant, vibrant flowers and numerous therapeutic properties.⁴⁻⁵ While historically used for its medicinal value, *S. asoca* has also contributed to the human diet, particularly through the consumption of its flowers, which are rich in bioactive compounds. These flowers not only offer antioxidant benefits but also enhance the color, flavor, and aroma of foods and beverages.⁶⁻⁷

Beyond their role as a food additive, the phytochemicals found in the flowers—flavonoids, glycosides, Saponins, and phenols- have been linked to a wide spectrum of health benefits, from antibacterial activity to oxytocic effects.⁸⁻¹⁰ The plant is a key component of Ayurvedic therapies for menstrual

disorders, fever, rheumatism, and various gynaecological issues.¹²⁻¹⁴ It also holds sacred significance, being used in religious ceremonies and symbolic of devotion in Hindu rituals.¹³

Despite its deep-rooted importance in traditional medicine, there has been limited exploration into the comparative analysis of its phytoconstituents across different solvent-based extractions, especially considering how solvent polarity influences bioactive compound yields. This study aims to bridge the gap by using Gas Chromatography-Mass Spectrometry (GC-MS) to profile crude flower extracts prepared in aqueous, ethanol, and hydroalcoholic solvents. Through this, we provide a novel dataset that highlights solvent-dependent variations in the phytochemical composition of *S. asoca* flowers, offering new insights into its medicinal potential and paving the way for targeted therapeutic applications.

Material & Method:

Collection and authentication of plant material

"*Saraca asoca*" flowers (Fig.1) were carefully harvested from the lush campus of Banaras Hindu

*Author for Correspondence: kumarsanjeevdg@bhu.ac.in

University (BHU), located at GPS coordinates 25°01'04.3608" N and 82°59'25.7784" E, Varanasi. The plant's authenticity was rigorously verified by the Department of Dravyaguna, ensuring its accurate identification and medicinal relevance (Figure 1). A voucher specimen, bearing the number DG/23-24/813, has been formally cataloged and deposited at the Dravyaguna Museum within the Institute of Medical Sciences (IMS), BHU, Varanasi, Uttar Pradesh, preserving the sample for future reference and research.

Preparation and extraction of plant material

Fresh flowers of *Saraca asoca* were hand-harvested and shade-dried at ambient temperature over several days, ensuring preservation of their delicate bioactive compounds and preventing any premature degradation. After drying, the flowers were meticulously ground into a coarse powder using a traditional mortar (*Khalwa-yantra*), a tool of Ayurvedic significance, which enhances the powder's consistency and maintains its medicinal integrity. The powdered material was then stored in airtight containers at room temperature to safeguard against moisture and oxidation.

For the extraction process, 200 g of the dried flower powder was subjected to solvent extraction using three different solvent systems: alcoholic, aqueous, and a hydroalcoholic mix (3:7). Each sample was gently agitated for 24 hours on an orbital shaker to ensure thorough extraction of phytochemicals. Following extraction, the mixture was filtered through a Büchner funnel with Whatman No. 01 filter paper, effectively separating the plant residue from the solvent-based extract. The filtrates were then concentrated to dryness under reduced pressure using a rotary evaporator, yielding the respective extracts- alcoholic, aqueous, and hydroalcoholic ready for GC-MS analysis to reveal the chemical profile of the flowers.

GC-MS (Gas Chromatography-Mass Spectrometry) analysis

Gas chromatography-mass spectrometry (GC-MS) analysis was performed on the alcoholic, aqueous, and hydro-alcoholic extracts of *Saraca asoca* flowers using a Perkin Elmer GC-MS [Model Clarus 680 GC] system equipped with a fused silica capillary column (TR-5MS, 30 m × 0.25 mm × 0.25 mm ID). Helium served as the carrier gas at a constant flow rate of 1.2 mL/min. The injector temperature was maintained at 250°C throughout the analysis. Each extract (1.0 µL) was individually injected into the system. The oven temperature program was set as follows: initial temperature of 40°C held for 4 minutes, ramped to 100°C and held for 4 minutes, further increased to 200°C for 4 minutes, and finally raised to 280°C and held for another 4 minutes, resulting in a total runtime of 16 minutes. The mass detector system included both a Flame Ionization Detector (FID) and DMSO as

detectors 1 and 2, respectively. The transfer line and ion source temperatures were maintained at 250°C and 200°C. The ionization was carried out using electron impact (EI) mode at 70 eV. The scan time was set to 0.5 seconds with an inter-scan delay of 0.1 seconds, and the detector operated in scan mode over a mass range of 30–500 amu.

Result & Discussion:

In the present study, a comprehensive analysis of the bioactive compounds present in *Saraca asoca* flowers was performed using Gas Chromatography-Mass Spectrometry (GC-MS), revealing a diverse array of secondary metabolites across three solvent extracts: aqueous, ethanolic, and hydroalcoholic. This solvent-based approach highlights previously unexplored differences in the composition of phytochemicals and their potential biological activities.

The aqueous extract revealed three important compounds: 2-Methyl-glycerol, Malvidin, and Peonidin (Fig. 2). 2-Methyl-glycerol (C₄H₁₀O₃) is a glycerol derivative with notable hydrophilic properties, enhancing its potential in aqueous formulations. Malvidin (C₁₇H₁₅O₅), a prominent anthocyanin in red grapes and wine, is known for its anti-inflammatory, anti-carcinogenic, anti-diabetic, and cardioprotective effects.¹⁶⁻²⁰ Similarly, Peonidin (C₁₆H₁₅O₆), another anthocyanin, provides anti-inflammatory, antioxidant, and cardioprotective properties, while also serving as a natural coloring agent.²¹⁻²⁵

The ethanolic extract revealed Quercetin (C₁₅H₁₁O₆), Delphinidin (C₁₅H₁₁O₇), Bisabolane-type sesquiterpenoids, and Kaempferol dianion (Fig. 3). Quercetin, a flavonoid widely distributed in fruits and vegetables, exhibits a broad range of health benefits, including anti-carcinogenic, antidiabetic, and antioxidant activities [26-28]. Delphinidin, a natural anthocyanin, has demonstrated antioxidant, anticancer, and anti-inflammatory effects, with applications as a natural colorant.²⁹⁻³² The presence of Bisabolane-type sesquiterpenoids (C₁₅H₂₈O₄), known for their antibacterial, anti-inflammatory, and cytotoxic properties, adds novel insights into the bioactivity of *S. asoca*.³³⁻³⁴

The hydroalcoholic extract presented Kaempferol (C₁₅H₁₀O₆), Luteolin (C₁₅H₁₀O₆), and the Kaempferol tetra-anion (C₁₅H₆O₆) (Fig. 4). Kaempferol is a widely studied flavonoid with established anti-inflammatory, cardioprotective, and antioxidant effects [36-37]. Luteolin, another flavonoid, has shown anti-inflammatory, antimutagenic, and cardioprotective effects [38], while the unique Kaempferol tetra-anion, formed under highly alkaline conditions, may have altered solubility and stability properties, offering a new avenue for research into the solubility profiles of flavonoids in mixed solvent systems.

This study represents a novel contribution to the understanding of the solvent-dependent extraction of

bioactive compounds from *Saraca asoca* flowers. By comparing the chemical profiles from different solvents, we provide insights into the variability of bioactivity linked to solvent polarity and extraction conditions. These findings open new doors for optimizing extraction methods and advancing the use of *S. asoca* in pharmaceutical, nutraceutical, and cosmetic applications. Future studies focused on isolating these compounds and evaluating their biological activities, pharmacokinetics, and toxicity profiles could significantly enhance the therapeutic potential of this traditional plant.

Conclusion:

This study presents a novel comparative analysis of the phytochemical composition of *S. asoca* flowers using solvent-dependent extraction methods and Gas Chromatography-Mass Spectrometry (GC-MS). For the first time, solvent-specific profiles were uncovered, revealing distinct bioactive compounds such as 2-Methyl-glycerol, Malvidin, Peonidin, Quercetin, Delphinidin, Bisabolane-type sesquiterpenoids, and Kaempferol derivatives across aqueous, ethanolic, and hydroalcoholic extracts. These findings demonstrate the solvent's pivotal role in selectively extracting bioactive metabolites and their subsequent bioactivity, offering insights into the plant's therapeutic potential. The discovery of Kaempferol tetra-anion in the hydroalcoholic extract and Bisabolane-type sesquiterpenoids in the ethanolic extract introduces previously unreported compounds, which enrich the phytochemical profile of *S. asoca* and underscore the untapped potential of this plant for pharmaceutical, nutraceutical, and cosmetic applications. The observed solvent-specific bioactivity from anti-inflammatory and anticancer effects to antioxidant and cardio-protective properties opens new pathways for the targeted use of *S. asoca* in modern drug discovery. This research provides a foundation for the optimization of extraction methods and drug development using *S. asoca*, laying the groundwork for future studies to isolate and evaluate the individual bioactive compounds for their pharmacological activities, toxicity profiles, and commercial applications. By addressing the solvent dependency and highlighting key bioactive molecules, this study contributes to a deeper understanding of the plant's medicinal value and its potential integration into the evolving field of natural product-based therapeutics.

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Author's contribution:

Sanjeev Kumar and Karthika Murukan drafted the original manuscript. Karthika Murukan collected the raw drug and formed the different extracts. Ankita Yadav contributed to the data curation, design, and data analysis of the manuscript. GC-MS analysis was performed by Poonam Pal under the supervision of S. Krishnamoorthi. Komal Jayram Wadekar and Anurag Mishra assisted with editing and preparation of the final version of the manuscript. All authors read and approved the final version of the manuscript.

Availability of data and materials

Data from this study will be available upon reasonable request to the corresponding author.

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Consent to participate

Not applicable. This study did not involve human participants.

Consent to publish

Not applicable. This study did not involve human participants or personal data requiring consent for publication

Competing interests

The authors declare that they have no competing interests.

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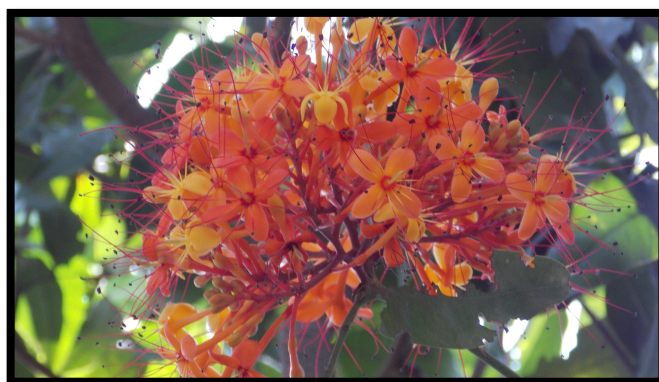

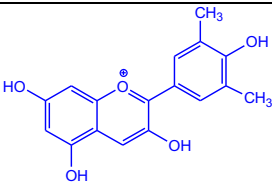
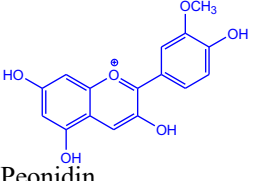
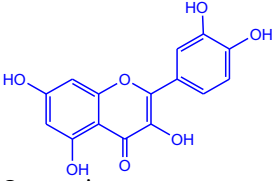
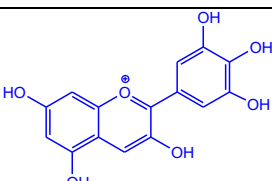
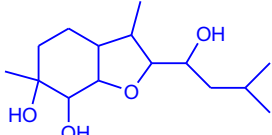


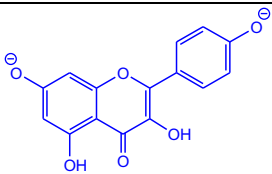
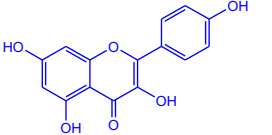
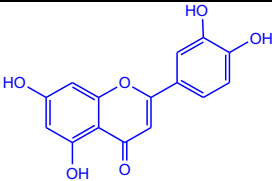
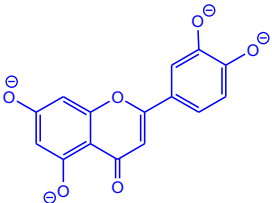
Fig. 1: Flower of *Saraca asoca* (Roxb.) Wild.

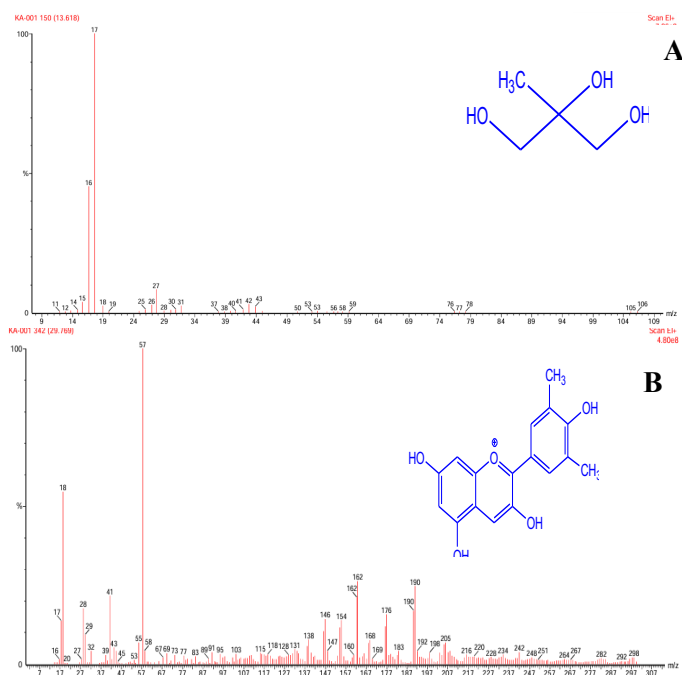
Table No. 1: Showing the components detected in different solvent extracts of *Saraca asoca* flower-

Peak	Mol. Formula	Mol. weight	Base peak	Name of the compound	Bioactivity of the compound
1.	C ₄ H ₁₀ O ₃ Smile: COC(CO)CO	106	17	2-Methyl-glycerol 	Antioxidant activity Anti-ulcerative Anti-inflammatory Antidiabetic activity Antihypertensive Anticarcinogenic Anti-cataract activity

2.	C ₁₇ H ₁₅ O ₅ Smile: <chem>[Cl].COc1cc(cc(OC)c1O)c2[o+]c3cc(O)cc(O)c3cc2O</chem>	300	57	 <p>Malvidin (Anthocyanines)</p>	Anti-inflammatory Anticarcinogenic Antidiabetic activity Cardio-protective effect Dementia Treatment
3.	C ₁₆ H ₁₃ O ₆ Smile: <chem>[Cl].COc1cc(ccc1O)c2[o+]c3cc(O)cc(O)c3cc2O</chem>	301	91	 <p>Peonidin (Anthocyanines)</p>	Antioxidant Anti-inflammatory Anti-cancerous activity Prebiotic activity
4.	C ₁₅ H ₁₁ O ₆ Smile: <chem>Oc1cc(O)c2C(=O)C(=C(Oc2c1)c3cc(O)c(O)c3)O</chem>	302	18	 <p>Quercetin (Flavones)</p>	Antioxidant activity Antiseborrheic Anticarcinogenic Antiinflammatory Antihypercholesterolemic Anti-leukemic Anti- tuberculosis Laxative activity Gout treatment Antibacterial Antiuremic
5.	C ₁₅ H ₁₁ O ₇ Smile: <chem>[Cl-].Oc1cc(O)c2cc(O)c([o+]c2c1)c3cc(O)c(O)c(O)c3</chem>	303	18	 <p>Delphinidine (Anthocyanines)</p>	Neuroprotective effect Anti-carcinogenic Antiinflammatory Antioxidant activity
6.	C ₁₅ H ₂₈ O ₄ [M + Na] ⁺ Smile: <chem>CC(C)CCCC(C)C1CCC(C)CC1</chem>	295	18	 <p>bisabolane-type sesquiterpenoids</p>	Anti-eczematic activity Hypolipemic Anti-psoriatic Anti-metastatic Anti-seborrheic Anti-fungal activity Anti-osteoporotic Wound healing agent Antiulcerative activity Anti-cataract activity

“Solvent-Dependent Phytochemical Profiling of *Saraca asoca* Flowers: A Comparative GC-MS Study”

7.	$C_{15}H_8O_6$ Smile:	284	18	 <p>Kaempferol dianion (Flavonol)</p>	Antioxidant Antiinflammatory Hepatoprotectant Antibacterial Antidiabetic Antineoplastic
8.	$C_{15}H_{10}O_6$ Oc1ccc(cc1)C2=C(O)C(=O)c3c(O)cc(O)cc3O2	286	18	 <p>Kaempferol (Flavonol)</p>	Antimutagenic Anti-hemorrhagic activity Antioxidant Anti-inflammatory Hepatoprotectant (Influenza) Antiviral Antibacterial Antidiabetic Antineoplastic activity
9.	$C_{15}H_{10}O_6$ Smile: Oc1cc(O)c2C(=O)C=C(Oc2c1)c3c(O)c(O)c3	286	18	 <p>Luteolin (Flavones)</p>	Antimutagenic Antiseborrheic Antioxidant Anticarcinogenic Antiinflammatory Antiuremic Antiulcerative Antipyretic
10.	$C_{15}H_6O_6$	282	18	 <p>Kaempferol tetra-anion (Flavonol)</p>	Antioxidant Anti-inflammatory Anticancer Neuroprotection



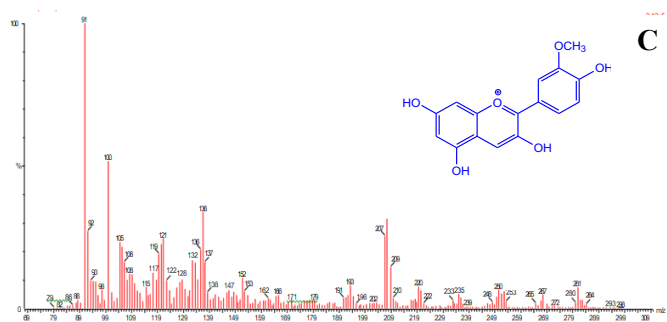
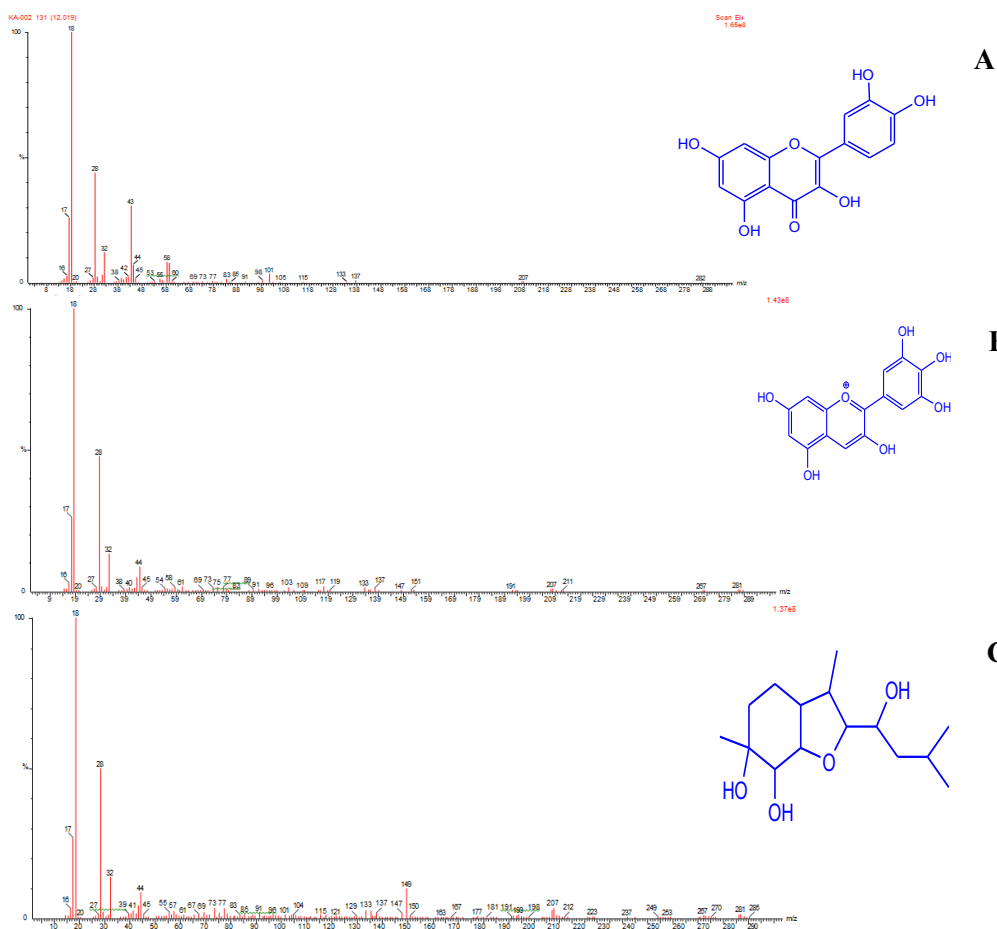


Fig. 2: GC-MS analysis of the aqueous extract of the flower of *Saraca asoca* showing (A) 2-Methylglycerol (B) Malvidin (C) Peonidin



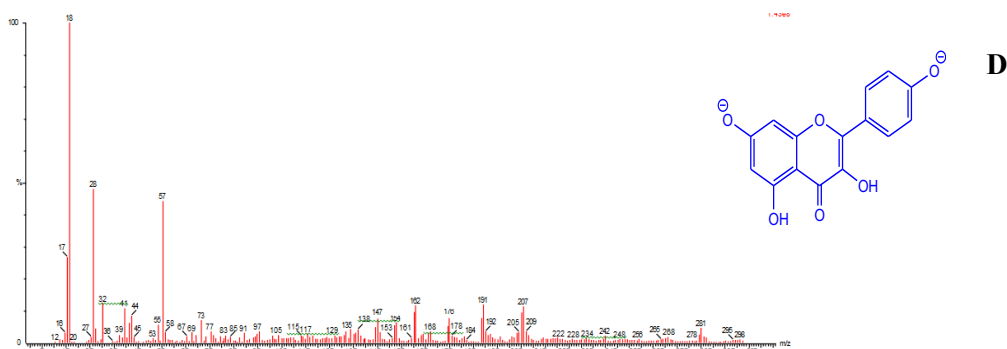


Fig. 3: GC-MS analysis of the ethanolic extract of the flower of *Saraca asoca* showing (A) Quercetin (B) Delphinidine (C) Bisaboloane- type sesquiterpenoids (D) Kaempferol dianion

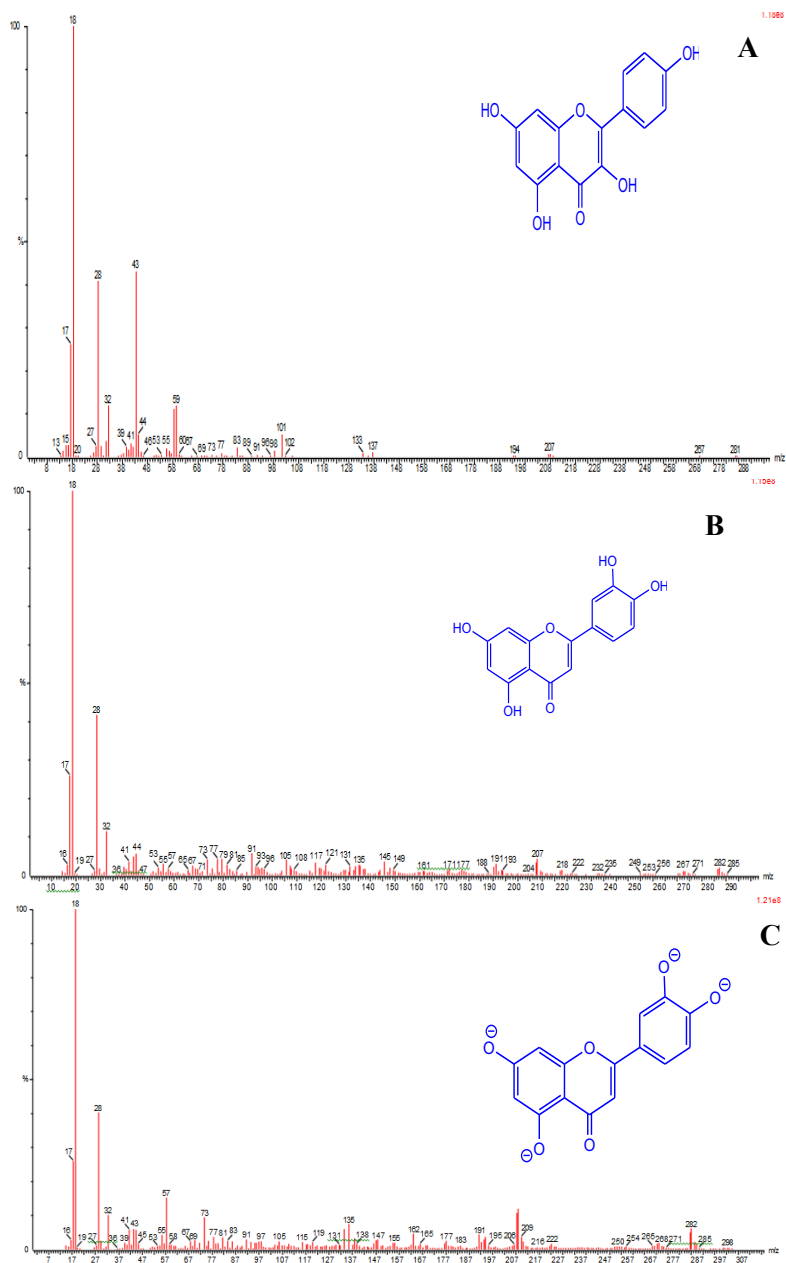


Fig. 4: GC-MS analysis of the Hydro- alcoholic extract of the flower of *Saraca asoca* showing (A) Luteolin (B) Kaempferol (C) Kaempferol tetra-anion