Original Article

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Ayurveda Section

Phytochemical Characterisation and Pharmacological Evaluation of *Vedanasthapana Gana* in Multiple Dosage Forms using HPTLC and GC-MS: An In-vitro Experimental Study

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ABSTRACT

Introduction: *Vedanasthapana Gana* is a classical Ayurvedic multiherbal formulation described by *Acharya Charaka* for relieving pain. It has been traditionally used in different painful conditions for many years. However, scientific data comparing its chemical composition in different dosage forms is limited. Modern analytical studies are needed to ensure its quality, consistency, and pharmacological applicability when prepared in various forms.

Aim: To analyse the phytochemical composition and pharmacological applicability of *Vedanasthapana Gana* prepared as oil, methanolic extract, and rectal suppository using physicochemical tests, High Performance Thin Layer Chromatography (HPTLC), and Gas Chromatography-Mass Spectrometry (GC-MS).

Materials and Methods: An in-vitro experimental laboratory-based study was carried out at the Centre of Research for Development Parul University, Vadodara, Gujarat, India and CARE KERALAM, Kerala, India from May 2023 to March 2025. *Vedanasthapana Gana* was prepared in three dosage forms: medicated oil, methanolic extract, and rectal suppository. Physicochemical parameters were assessed according to Ayurvedic Pharmacopoeia of India guidelines. HPTLC analysis was performed for all three forms using silica gel plates and scanned at 254 nm and 366 nm. GC-MS analysis was carried out for the methanolic extract using an Agilent GC-MS system to identify volatile and semivolatile compounds.

Results: *Vedanasthapana* oil showed prominent lipid-specific constants with slightly acidic pH (4.8), while the suppository was near basic (7.8) with higher ash content (1.13) and lower moisture (0.15%). The methanolic extract had greater viscosity, specific gravity, and total solids (1.70%) with acidic pH (4.5). Both oil and suppository tested negative for rancidity. HPTLC analysis revealed bioactive phytochemicals including gallic acid, ferulic acid, quercetin, kaempferol, luteolin, apigenin, β-sitosterol, and lupeol, with the extract showing the widest profile. GC-MS profiling of the extract identified sesquiterpenes, chalcone derivatives, and a steroidal compound associated with anti-inflammatory, analgesic, and antimicrobial activities.

Conclusion: The study confirms that *Vedanasthapana Gana* contains multiple pharmacologically active compounds in all three dosage forms. Differences in phytochemical profiles were observed due to variation in the method of preparation and dosage form. The use of HPTLC and GC-MS provided reliable scientific evidence for the presence of bioactive constituents, supporting the traditional use of *Vedanasthapana Gana* and its further development and standardisation in different dosage forms.

Keywords: Ayurvedic formulation, Gas Chromatography-Mass Spectrometry, High performance thin layer chromatography, Phytochemical profiling

INTRODUCTION

Vedanasthapana Gana is a classical Ayurvedic group of ten herbs mentioned by *Acharya Charaka* in the *Charaka Samhita Sutrasthana* [1]. The term "*Vedanasthapana*" literally means "pain-alleviating," and this formulation has traditionally been prescribed for conditions involving pain and inflammation. The herbs included in this group, such as *Shorea robusta*-*Shala*, *Myrica nagi*-*Katphala*, *Anthocephalus indicus*-Kadamba, and *Salmalia malabarica*-*Shalmali* are well known in Ayurvedic practice for their analgesic and anti-inflammatory properties. An animal experiment study conducted on ethanolic extract of *Shorea robusta* resin showed that the extract produced significant central and peripheral analgesic effects, as is evident from increase in reaction time in hot plate and tail flick tests, inhibition in writhing counts in acetic acid-induced writhing test, inhibition of licking time in formalin-induced hind paw licking, increased pain threshold in paw withdrawal latency in carrageenan-induced hyperalgesia and increased paw withdrawal threshold in post-surgical pain [2]. Also, aqueous extract of *Anthocephalus cadamba* showed significant reduction in the number of writhing induced by acetic acid and increased reaction time in hot plate test [3]. These studies have confirmed that these plants contain bioactive compounds like flavonoids, phenolic acids, and triterpenoids, which are capable of reducing pain, inflammation, and oxidative stress. Varnale GS and Varnale RG, observed that *Vedanasthapana gana* exhibited an analgesic effect at test doses of 50 mg/kg and 100 mg/kg, which may be mediated by central receptors. The onset of its analgesic effect after administration was seen at 30 minutes, which peaked at 60 minutes and lasted beyond 90 minutes. It could be recommended as a supplemental add-on to drugs used to manage chronic pain conditions like arthritis. It may help to decrease the dose and associated dose-dependent adverse effects of other drugs [4].

Despite its long-standing use, scientific validation of *Vedanasthapana Gana* remains limited. Most studies have focused on single dosage forms or preliminary pharmacological testing, leaving significant gaps in understanding its full potential [5]. There is a lack of systematic phytochemical profiling across different preparations, and the combined mechanism of action of the formulation as a whole has not been adequately explored. Furthermore, while Ayurveda traditionally prescribes decoctions and oils, newer dosage forms such as rectal suppositories have not been scientifically evaluated,

even though they may offer unique therapeutic advantages. This represents a significant research gap, as changes in formulation can influence extraction efficiency, stability, and availability of bioactive constituents.

Another major limitation in existing literature is the lack of comparative analytical fingerprinting of multiple dosage forms of a single Ayurvedic formulation using advanced techniques. Modern analytical tools like HPTLC and GC-MS play a crucial role in identifying marker compounds and establishing reproducible quality standards [6,7].

Hence, the present study was designed to evaluate *Vedanasthapana Gana* in oil, methanolic extract, and rectal suppository forms through physicochemical analysis, HPTLC fingerprinting, and GC-MS profiling. By comparing the phytochemical composition across these dosage forms, the study aimed to generate scientific evidence supporting the formulation's mechanism of action, standardisation, and relevance in pain management, thereby contributing to the integration of classical Ayurvedic formulations with modern pharmaceutical science.

MATERIALS AND METHODS

The present in-vitro experimental, analytical laboratory-based study was conducted between May 2023 and March 2025 at Parul University, Vadodara, Gujarat, India for HPTLC analysis, and at CARE KERALAM, Koratty, Kerala, India for GC-MS analysis, after obtaining institutional ethical clearance (PU/PINIEC/07/2023/292). Ethical Clearance certificate number is PU/PINIEC/07/2023/292 Dated 03/04/2023. Drugs of *Vedanasthapana Gana* [Table/Fig-1], sesame oil, paraffin, were procured from a local vendor and cocoa butter was procured online from a licensed vendor.

S. No.	Drugs	Botanical name	Family	Part used
1	*Shala*	*Shorea robusta* L.	Dipterocarpaceae	Resin
2	*Katphala*	*Myrica nagi* L.	Myricaceae	Stem bark
3	*Kadamba*	*Anthocephalus indicus* L.	Rubiaceae	Stem bark
4	*Padmaka*	*Prunus cerasoides* L.	Rosaceae	Hard wood
5	*Tumba*	*Langenaria siceraria* L.	Cucurbitaceae	-*
6	*Shalmali*	*Salmalia malabarica* L.	Bombacaceae	Stem bark
7	*Shirisha*	*Albizzia lebback* L.	Leguminoseae	Stem bark
8	*Vanjula*	*Salix tetrasperma* L.	Salicaceae	-*
9	*Elavaluka*	*Prunus avium* L.	Rosaceae	-*
10	*Ashoka*	*Sarraca Ashoka* L.	Leguminosae	Stem bark

[Table/Fig-1]: Ingredients of *Vedanasthapana gana.*
*Here we excluded Tumba, Vanjula, Elavaluka as there are controversies regarding their identification

All the raw materials were authenticated at the Department of *Dravyaguna*, Parul Institute of Ayurved, Parul University by the head of the department. The certificate number is PU/PIA/certi-242.

Vedanasthapana oil, suppository and extract was prepared in GMP certified pharmacy at Parul Institute of Ayurved & Research, Vadodara, Gujarat, India.

Preparation of *vedanasthapana* formulations

Vedanasthapana Oil was prepared according to *Taila Paka Vidhi* described in the Ayurvedic Formulary of India (AFI) [8]. All ingredients were taken in equal quantity, coarsely powdered, and boiled with 16 parts of water until reduced to one-fourth. The filtrate was then mixed with an equal quantity of sesame oil and heated until *Samyak Taila Paka Pareeksha* was achieved. The final oil was filtered and used for further processes [Table/Fig-2a].

Vedanasthapana Rectal Suppositories were prepared following standard pharmaceutics protocol. Cocoa butter and paraffin wax were melted on a water bath, after which the prescribed quantity of oil was added and mixed thoroughly. Semisolid extract was incorporated, homogenised, and immediately poured into suppository moulds. After cooling for 15-20 minutes, the suppositories were collected, wrapped in aluminium foil, and stored in a cool chamber [Table/Fig-2b].

Vedanasthapana Extract was prepared according to Indian Pharmacopoeial standards. Coarse powders of the herbal drugs were subjected to alcoholic extraction in a Soxhlet apparatus using methanol (1 part drug: 10 parts solvent) at 35°C. A total of 18 extraction cycles were completed to obtain the extract. Resin was extracted separately and combined with the herbal extract. The mixture was filtered and evaporated on a water bath until a semisolid consistency was achieved [Table/Fig-2c].

[Table/Fig-2]: a) *Vedanasthapana* oil; b) *Vedanasthapana* suppository; c) *Vedanasthapana* extract.

The physicochemical standardisation of the samples was carried out in accordance with the API for the parameters like organoleptic and physiochemical properties.

High-performance thin-layer chromatography Analysis: Chromatography was performed on 10 × 10 cm TLC plates coated with a 0.2 mm layer of silica gel 60 F254 (Merck) on aluminium sheets. Samples were applied to the plates as 6 mm wide bands using a Linomat 5 sample applicator (CAMAG, Switzerland). The plates were developed to a distance of 80 mm from the base using a mobile phase consisting of Toluene, Ethyl Acetate, and Methanol in a 9:1:1 v/v/v ratio within a CAMAG twin-trough chamber saturated with the mobile phase vapour. After drying, the plates were scanned post-derivatisation at 254 nm and 366 nm using a CAMAG TLC Scanner 3 with WinCATS 4 software (CAMAG, Switzerland).

Gas Chromatography-Mass Spectrometry Profile Procedure: Analysis was carried out by injecting 2 µL of the sample in split less mode. Helium gas (99.9995%) was utilised as carrier gas with a flow rate of 1 mL/min. Analysis was carried out in the Electron Impact (EI) mode using 70 eV of ionisation energy. The temperature of the injector was set at 280°C (constant). GC-MS analysis of the *Vedanasthapana* extract was performed using an Agilent Technologies GC-MS system comprising a 7890A gas chromatograph coupled with a 5975C mass selective detector equipped with a triple-axis detector. Separation was achieved on a DB-5MS capillary column (30 m × 0.25 mm internal diameter × 0.25 µm film thickness).

For sample preparation, the *Vedanasthapana* extract was filtered through a nylon syringe filter (13 mm, 0.2 µm pore size), transferred into a GC vial, and subjected to analysis. A volume of 2 µL of the filtered sample was injected in splitless mode. Helium (99.9995% purity) was employed as the carrier gas at a constant flow rate of 1 mL/min. Mass spectrometric detection was carried out in EI ionisation mode at an ionisation energy of 70 eV. The injector temperature was maintained at 280°C throughout the analysis. The oven temperature program was initiated at 40°C and held for five minutes, followed by a temperature ramp of 5°C/min up to 100°C with a hold time of 10 minutes. Subsequently, the temperature was increased at a rate of 20°C/min to a final temperature of 280°C and held for five minutes.

Identification of the phytochemical constituents was accomplished by interpretation of the mass spectra obtained from GC-MS analysis using the National Institute of Standards and Technology (NIST) mass spectral library, containing more than 62,000 reference

spectra. The mass spectra of the unknown components were compared with those of known compounds stored in the NIST 08 spectral database [9]. Based on spectral matching, the chemical name, molecular weight, and probable structure of the identified components in the test sample were confirmed.

RESULTS

Physicochemical standardisation: *Vedanasthapana* oil was golden yellow and oily with an astringent smell, while the suppository was creamish-brown, semi-solid, and uniform in form. The methanolic extract was dark brown with a strong bitter-astringent smell. The pH of oil (4.8) and the extract (4.5) is slightly acidic, whereas the suppository had near basic pH (7.8). Loss on drying was higher in oil (0.85%) than in the suppository (0.15%), meaning that there was a lesser amount of water in the suppository. The maximum value of ash content was found in suppository (1.13), followed by oil (0.02), and minimum in extract (0.003). Lipid-specific constants such as refractive index, acid value, iodine value, and saponification value were strongly evident in oil, and the values were relatively low in the suppository. Oil and suppository gave a negative result for rancidity. The methanolic extracts had higher values of viscosity, specific gravity, and total solid content (1.70%) than the other preparations [Table/Fig-3].

Sr No.	Parameters tested	*Vedanasthapana* Oil	*Vedanasthapana* Suppository	*Vedanasthapana* Extract
1	Colour	Golden yellow	Creamish brown	brown
2	Odour	Astringent	Astringent	Bitter astringent
3	Consistency	Liquid (oily)	Semisolid	Liquid
4	Shape	-	Torpedo shape	-
5	Size	-	2.9 cm	-
6	Diameter	-	0.8 cm	-
7	Loss on drying @110^{0}c (%w/w)	0.85	0.15	-
8	pH value	4.8	7.8	4.5
9	Ash value (%w/w)	0.02	1.13	0.003
10	Refractive index	1.4550	-	-
11	Specific gravity	0.9123	-	3.4751
12	Acid value	2.3	2.8	-
13	Iodine value	107	70	-
14	Saponification value	210	178	-
15	Rancidity test	Negative (not rancid)	Negative (not rancid)	-
16	Average weight (g)	-	3.04	-
17	Total solid content (% w/w)	-	-	1.70
18	Viscosity	24.21	-	87.977

[Table/Fig-3]: Physicochemical analysis of *Vedanasthapana Gana* Dravya.

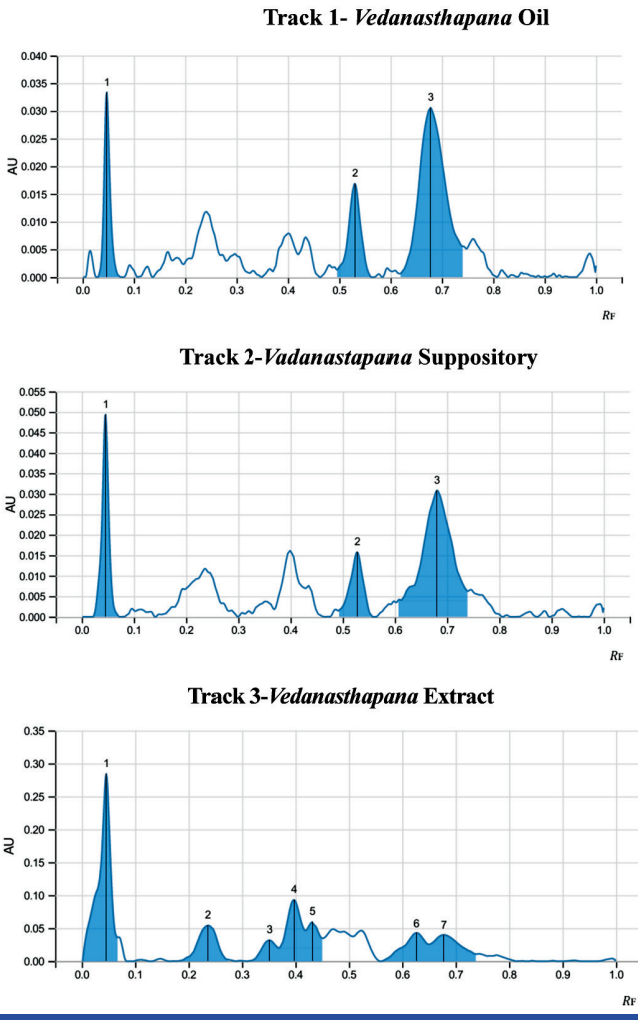
HPTLC fingerprinting: At 254 nm, *Vedanasthapana* oil and the suppository had closely similar Rf values of about 0.046-0.047 and 0.528-0.531, respectively, implying the co-presence of similar UV-absorbing compounds. The extract also had the same Rf value of approximately 0.678 as the oil and suppository. The methanolic extract showed other spots at Rf values of 0.236, 0.351, 0.397, 0.432, and 0.626 at wavelength 254 nm, which are absent in oil and at 366 nm, oil and suppository were equal with respect to Rf value (0.044); however, the value was slightly lower for the extract (0.025) [Table/Fig-4].

At 254 nm, multiple dark bands corresponding to UV-absorbing constituents were visible, with oil and suppository showing similar band positions, while the extract showed a higher number of bands at 366 nm, fluorescent bands are observed across all tracks, with variation in number and intensity among the oil, suppository, and extract, indicating differences in detectable compounds under long-wavelength UV light [Table/Fig-5-7].

Scanning wave lengths	Track 1 *Vedanasthapana* Oil	Track 2 *Vedanasthapana* Suppository	Track 3 *Vedanasthapana* Extract
At 254 nm	0.047	0.046	0.046
	0.531	0.528	0.236
	0.678	0.681	0.351
			0.397
			0.432
			0.626
			0.678
At 366 nm	0.044	0.044	0.025
		0.510	0.226

[Table/Fig-4]: Showing Rf values obtained from HPTLC fingerprint profile.

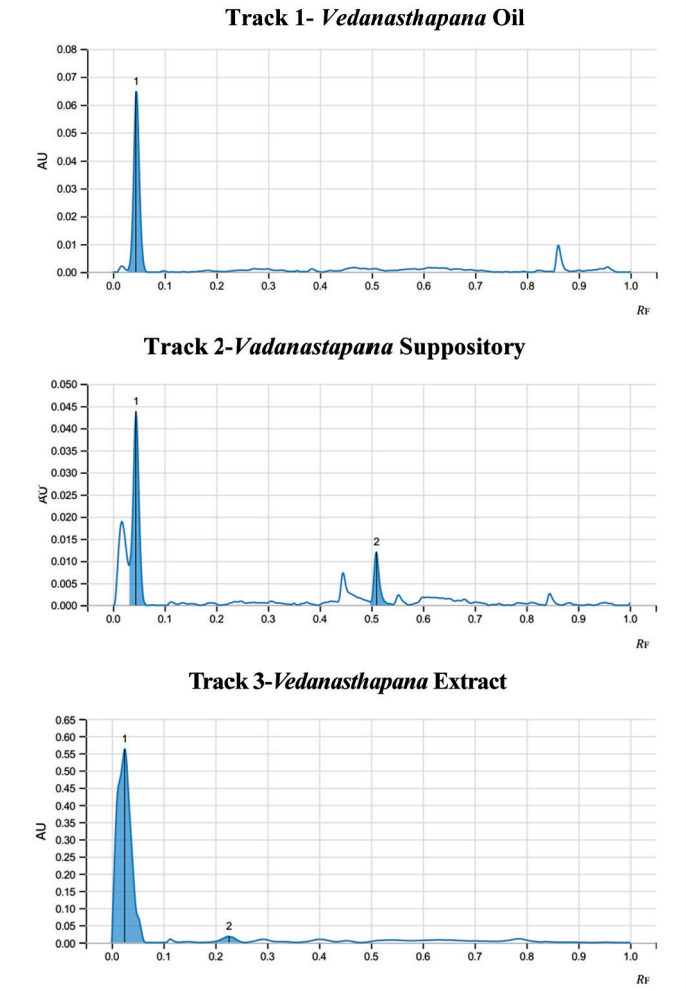
The resultant GC-MS chromatogram shows many resolved peaks, which are primarily eluting between ~28 and 36 minutes, and therefore suggest several semi-volatile phytoconstituents present in this sample. The major peaks that appeared around 31-34 minutes with higher abundance correspond to the dominant compounds, while the small peaks observed during later retention times suggest minor constituents. Overall, the chromatographic pattern reflects a complex chemical profile typical of the studied extract [Table/Fig-8].



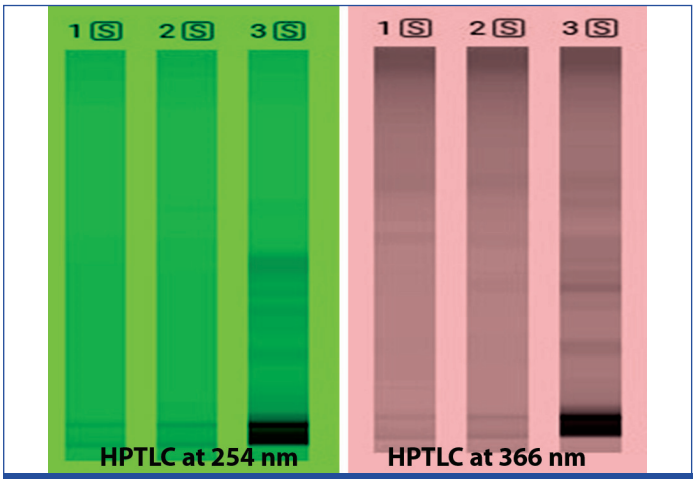
[Table/Fig-5]: Densitometric HPTLC fingerprint chromatogram of *Vedanasthapana* oil, suppository and extract recorded at 254 nm, showing major phytoconstituent peaks plotted as absorbance units (AU) versus Rf values.

DISCUSSION

The comprehensive analytical assessment of *Vedanasthapana Gana* across its three dosage forms i.e., oil, extract, and rectal sup-



[Table/Fig-6]: Densitometric HPTLC fingerprint chromatogram of *Vedanasthapana* oil, suppository and extract recorded at 366nm, showing major phytoconstituent peaks plotted as absorbance units (AU) versus Rf values.



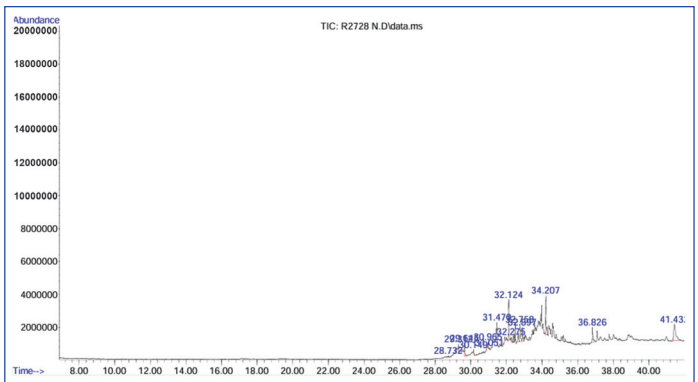
[Table/Fig-7]: HPTLC fingerprint profile of *Vedanasthapana* extract showing chromatographic bands of samples (S1-S3) visualised under UV light at 254 nm and 366 nm.

pository, showed a diverse range of phytoconstituents with strong pharmacological relevance, particularly in the context of pain and inflammation.

Physicochemical parameters confirmed the integrity and stability of the formulations. Differences in the properties of colour, smell, and consistency are due to the type of base employed and the level of concentration of the phytoconstituents, with the extract showing more properties due to solvent extraction. The near alkaline nature of the suppository, which is not like the mild acidity of *Taila* and extracts, is possibly due to the addition of excipients and is suitable for rectal use. The lower loss of drying of the suppository suggests a more stable compound with lower chances of microbial growth [10]. The higher value of ash for the suppository can be due to the addition of base substance, and the zero value for the extract was indicative of its purity. Lipid-related values have confirmed the oily nature of the *Taila*. Lower iodine and saponification values in the suppository show that the lipid's properties change when it is converted into a dosage form. The negative rancidity test of both suppository and oil depicts its sustainability against oxidation [11]. The elevated viscosity, specific gravity, and total solids of the methanolic extract signify the concentration of bioactive compounds, which is different for the *Taila* and suppository. The data established that despite the same formulation, each of the dosage forms possesses a different physicochemical property that could affect stability, routes of delivery, and performance.

HPTLC fingerprinting profile clearly shows both similarities and dissimilarities in the phytochemical content of *Vedanasthapana Gana* in various forms of preparation. All dosage forms exhibited multiple pharmacologically active markers, including phenolic acids such as gallic acid [11], Ferulic acid [12], Vanillic acid [13], and contribute to antioxidant and free radical scavenging activity, further supporting anti-inflammatory pathways. The extract mainly retains flavonoids and selected phenolic compounds like apigenin [14] and luteolin [15] are also known to relax smooth muscles and help in pain modulation. Suppository form depicts Flavonoids such as Quercetin [16], Kaempferol [17] which have demonstrated analgesic and anti-inflammatory properties. The consistent detection of triterpenoids like β-Amyrin acetate [18] and Lupeol [19] in all dosage forms shows the formulation's analgesic effect. Therefore, the HPTLC pattern shows that the oil and the suppository preparation share a common phytochemical makeup with a lipid-soluble dominant showing and the methanolic extract has a diverse makeup [Table/Fig-9] [11-23].

GC-MS analysis of the extract showed a rich chemical profile dominated mainly by sesquiterpenes, along with a few oxygenated sesquiterpenes, diterpene derivatives, flavonoid-type compounds, and a steroidal compound [Table/Fig-8]. Most of the identified compounds belong to the sesquiterpene class, such as α-guaiene [24], copaene [25], α-amorphene [26], β-cubebene [27], cadina-1(10),4-diene [28]. These compounds are well known for their anti-inflammatory and antimicrobial activities. A significant finding is the presence of oxygenated sesquiterpenes, especially spathulenol [29], viridiflorol [30], and α-cadinol [31], which showed relatively higher peak intensities having majorly anti-inflammatory activity. Oxygenated terpenes are generally more biologically active than non oxygenated ones [32]. The detection of azulene derivatives is important, as azulenes are known for their anti-inflammatory and antispasmodic effects [33]. Overall, the GC-MS results indicate that the extract contains multiple bioactive compounds acting together. The combined presence of anti-inflammatory, antioxidant, antimicrobial, and antispasmodic compounds supports the therapeutic usefulness of the extract in pain disorders [Table/Fig-10] [24-33].



[Table/Fig-8]: Total Ion Chromatogram (TIC) obtained from GC-MS analysis of *Vedanasthapana* methanolic extract showing the distribution of detected phytoconstituents over retention time.

Rf value	Detected in track (s)	Probable compound	Phytochemical class	Probable plant source(s)	Probable pharmacological action
0.025	Track 3 @ 366 nm	Gallic acid	Phenolic acid	*Ashoka, Kadamba*	Anti-inflammatory, antioxidant [11]
0.044	Track 1, 2 @ 366 nm	Ferulic acid	Phenolic compound	*Padma*	Analgesic, hepatoprotective [12]
0.046	Track 1, 2, 3 @ 254 nm	Vanillic acid	Aromatic acid	*Shal, Padma*	Antimicrobial, pain-modulating [13]
0.226	Track 3 @ 366 nm	Catechin	Flavonoid	*Shal, Ashoka, Katphala*	Antioxidant, vasodilatory [20]
0.236	Track 3 @ 254 nm	Chlorogenic acid	Polyphenol	*Katphala, Padma*	Anti-inflammatory, analgesic [21]
0.351	Track 3 @ 254 nm	Apigenin	Flavone	*Ashoka, Kadamba*	Spasmolytic, anti-dysmenorrheal [14]
0.397	Track 3 @ 254 nm	Luteolin	Flavonoid	*Kadamba, Shirish*	Anti-inflammatory, estrogenic modulation [15]
0.432	Track 3 @ 254 nm	β-Sitosterol	Phytosterol	*Katphala*	Anti-inflammatory, hormonal balance [22]
0.510	Track 2 @ 366 nm	Quercetin	Flavonoid	*Ashoka, Padma*	Anti-inflammatory, uterine tonic [16]
0.528	Track 2 @ 254 nm	Kaempferol	Flavonoid	*Ashoka, Kadamba*	Analgesic, antioxidant [17]
0.531	Track 1 @ 254 nm				
0.626	Track 3 @ 254 nm	Stigmasterol	Phytosterol	*Shirish, Katphala*	Anti-inflammatory, oestrogen modulator [23]
0.678	All Tracks @ 254 nm	β-Amyrin acetate	Triterpenoid	*Katphala, Kadamba*	Anti-inflammatory, uterine relaxant [18]
0.681	Track 1, 2 @ 254 nm	Lupeol	Triterpenoid	*Kadamba, Katphala*	Anti-inflammatory, antispasmodic, analgesic [19]

[Table/Fig-9]: HPTLC peak profiling with phytochemical class, source, and probable action [11-23].
Rf values are interpreted based on standard HPTLC references, Ayurvedic Pharmacopoeia of India (API), and compound-specific monographs.

S. No.	Retention time (min)	Compound name	Peak height	Functional group(s)	Biological activity (reported/likely)
1	28.73	α-Guaiene	63,024	Sesquiterpene (alkene, hydrocarbon)	Anti-inflammatory, antimicrobial [24]
2	29.31	Copaene	416,593	Sesquiterpene (alkene)	Antioxidant, anti-inflammatory [25]
3	29.65	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis (1-methylethenyl)-	774,488	Hydrocarbon with alkenyl side chains	Possible fragrance, antimicrobial
4	30.15	Bicyclo (7.2.0) undec-4-ene, 4,11,11-trimethyl-8-methylene-, {1R-(1R*,4Z,9S*)}-	230,652	Bicyclic sesquiterpene	Anti-inflammatory, possibly analgesic
5	30.97	α-Amorphene	356,831	Sesquiterpene	Anti-inflammatory [26]
6	31.05	β-Cubebene	91,850	Sesquiterpene hydrocarbon	Antimicrobial, insecticidal [27]
7	31.47	Cadina-1(10),4-diene	1,270,140	Sesquiterpene diene	Antioxidant, anti-inflammatory [28]
8	32.12	Spathulenol	2,487,895	Sesquiterpene alcohol	Anti-inflammatory, antimicrobial [29]
9	32.27	Viridiflorol	233,257	Oxygenated sesquiterpene	Antibacterial, anti-inflammatory [30]
10	32.76	α-Cadinol	1,075,077	Sesquiterpene alcohol	Antioxidant, antifungal [31]
11	32.90	Azulene, 7-isopropyl-1,4-dimethyl-	860,173	Aromatic hydrocarbon	Anti-inflammatory, antispasmodic [32]
12	34.21	Platambin	2,364,310	Diterpene derivative	Anti-inflammatory, antimicrobial [33]
13	36.83	Pinostrobin chalcone	924,932	Chalcone (flavonoid class)	Anti-inflammatory, antioxidant, anti-ulcer
14	41.43	4,10-(Methanoxymethano)-10H-cyclopenta(a) phenanthren-3(4H)-one, 17-(acetyloxy)-1,2,7,8,9,11,12,13,14, 15,16,17-dodecahydro-13-methyl-, {4S-(4α,8α,9β,10α,13α,14β,17α)}-	950,124	Steroid nucleus, ester, oxygenated groups	Hormonal modulator, anti-inflammatory, potential adaptogenic effects [33]

[Table/Fig-10]: The retention values, the types of possible compound, peak height, their functional group and medicinal roles of each compound of the GC–MS profile of *Vedanasthapana* extract [24-33].

Limitation(s)

The present study had certain limitations. It was primarily a laboratory-based analytical investigation and did not include in-vivo or clinical evaluation to confirm therapeutic efficacy. In the HPTLC analysis, reference standards or controls were not run alongside the samples; therefore, compound identification was based on Rf values and literature correlation rather than direct standard comparison. In addition, the HPTLC results were interpreted qualitatively and statistical analysis for quantitative parameters such as peak areas, peak intensities, or relative concentration of phytoconstituents was not performed. GC-MS analysis was also restricted to the methanolic extract, while the oil and suppository were not evaluated using this technique.

CONCLUSION(S)

This study demonstrated that *Vedanasthapana Gana* prepared as oil, methanolic extract, and rectal suppository, retained core bioactive constituents with acceptable physicochemical stability and formulation-specific characteristics. HPTLC and GC-MS analyses confirmed the presence of phytochemicals with analgesic and anti-inflammatory potential, supporting its role in standardised pain management. Future studies should focus on safety, efficacy, bioavailability, and advanced analytical standardisation to establish its clinical utility.

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