Phytochemical Profiling of the Aqueous Methanolic Extract of the Traditional Polyherbal Product Batrisu Vasanu Using GC – MS

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Abstract: Batrisu vasanu is an ethnobotanically practiced nutraceutical believed to increase lactation and benefit new mothers in postpartum recovery. This mixture of 32 herbs is polyherbal in nature and requires the phytochemical profiling to better understand its clinical effect. With an aim to profile the phytochemicals, GC-MS was performed with aqueous methanolic extract of this polyherbal mixture. The sample showed various bioactive compounds present in the mixture. The GC-MS analysis revealed the presence of twenty-two phytochemicals namely Trifluoromethyl t-butyl disulfide, L-(+)-Ascorbic acid 2,6-dihexadecanoate, Bromoacetic acid, 2-ethylhexyl ester, and Farnesol isomer-A. Peak 5 at retention time 19.741 showed highest percentage area under the peak that was 22.742. The present study with phytochemical profiling of Batrisu vasanu revealed the presence of numerous bioactive compounds with effective medicinal properties. It can be concluded that the medicinal effect of Batrisu vasanu is due to these key bioactive phytochemicals.

Keywords: *Batrisu Vasanu; Galactagogue; Phytochemicals; GC-MS; Polyherbal.*

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I. INTRODUCTION

Plants are key source of medicines from ancient times for mankind (Morvin Yabesh et al., 2014). Potentials of herbs are explored and established traditionally among various cultures. Due to presence of numerous bioactive compounds, pharmaceutical industries are actively engaging in traditional natural products research (Malik et al., 2015; Pan et al., 2011).

Postpartum recovery for new mothers is an important clinical and social aspect that concerns researchers. Ethnobotanical remedies from diverse cultural traditions have been traditionally employed to support maternal health, facilitate postpartum recovery, enhance wound healing, mitigate blood loss, promote lactation, and contribute to the growth and development of infants (Ghasemi et al., 2015; Nordeng & Havnen, 2004; Watcho et al., 2019). These nutraceutical remedies are also believed to aid in the hormonal and anatomical restoration of the maternal reproductive system. Women used Fenugreek, Asparagus, Garden cress, Dill seeds, Galega, Milk thistle and ginger to improve lactation (Thangliankhup et al., 2022; Zuppa et al., 2010). Apart from the monoherbal product, a popular polyherbal product Batrisu vasanu, is reported from Gujarat, consumed by new mothers postpartum as galactagogue (Sanket et al., 2025). It is believed to improve health of mothers and aid in growth and development of infants. Batrisu vasanu is mixture of 32 herbs, powdered and mixed with wheat flour, sugar and ghee, and is prepared as sweet delicacy. It is consumed daily for up to two months postpartum starting from the second week of child delivery. Clinical information on this polyherbal mixture is lacking but phytochemical studies are warranted before clinical studies. Gas chromatography – Mass spectroscopy (GC-MS) is a sophisticated analytical technique that helps in determination and identification of phytochemicals (Kumar et al., 2011). It plays an essential role in clinical studies and chemotaxonomic studies of medicinal plants. With an aim to profile the

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phytochemicals in this novel polyherbal mixture, this study focuses on GC-MS based screening of bioactive compounds.

II. MATERIALS AND METHODS

➢ *Chemicals and Reagents*

For the present study, chemicals and reagents of Analytical grade were procured from SRL chemicals. The reagents were prepared fresh and otherwise stored at 4ºC.

➢ *Plant Materials*

Batrisu vasanu was prepared as mentioned previously (Charola et al., 2021). In brief, the plant materials were obtained from the market and were validated using literature.

➢ *Methanolic Extract Preparation*

The plant materials were dried in hot air oven for 24 hours at 40ºC. The dried plant parts were powdered in grinding mill and were passed through sieve size No. 40. The powder was stored in an air-tight container till further use. Then 10 g coarse powder was added to 100 ml methanol to obtain 10% extract. Extract was prepared by swirling the flask occasionally for 24 hours. Using rotary evaporator, the extract was concentrated and stored at 4ºC (Sanket et al., 2025).

➢ *GC-MS Analysis*

Using Agilent 7890A GC system coupled with 5675C inert equipped with triple-axis detector, GC-MS analysis was performed. The setup included an HP-5 MS fused silica capillary column (30.0 m × 250 µm i.d., 0.25 µm film thickness; 5% phenyl methyl siloxane). Helium served as the carrier gas, maintained at a constant flow rate of 1.0 ml/min (Olivia et al., 2021).

Instrumental parameters were configured as follows: ion source temperature at 250 °C, interface temperature at 300 °C, system pressure at 16.2 psi, and an out time of 1.8 mm. Sample injection was carried out in split mode (1 µl volume) with a split ratio of 1:50, and the injector temperature was set to 300 °C. The column oven was initially held at 36 °C for 5 minutes, ramped to 150 °C at 4 °C/min, then further increased to 250 °C at 20 °C/min and held for 5 minutes, resulting in a total run time of 40 minutes.

➢ *Phytochemical Identification*

Compound quantification was based on the relative percentage of each peak area compared to the total chromatographic area. Data acquisition and system control were managed using proprietary MS Solution software provided by the instrument manufacturer. NIST libraries, retention time and mass spectrum could lead to proper chemical identification (Yarazari & Jayaraj, 2022).

For obtained peaks, Retention time (RT) and corresponding Area and % Area for the peak was calculated as presented in Table 1. Among the 22 peaks, highest peak was observed for Comp 5, that represented 22.742 % peak area. It was followed by Comp 17 at RT 34.927 min, and 13.524 % area. Followed by Comp 9, Comp 3, and Comp 20 showed distinct peak at RT 27.119, 9.967, and 36.893 respectively.

Out of obtained chromatogram, four major peaks with highest % area under the curve were selected and were analyzed using m/z ratio in Mass spectroscopy.

III. RESULTS AND DISCUSSION

➢ *Gas Chromatogram*

Polyherbal mixture subjected to Gas chromatography was allowed to run upto 40 minutes. The results as shown in Fig. 1, were analyzed for the numbers of the peak, peak area, percentage (%) peak area, and retention time (RT). The result showed 22 peaks in the chromatogram as labelled in the diagram for their individual retention times.

The study revealed a good number of phytochemicals as it is a polyherbal product. The presence of these phytochemicals in methanolic extract of mixture indicates its numerous medicinal properties such as anti-microbial, anti-cancer, anti-obesity, wound healing and anti-diabetic, among others (Olivia et al., 2021).

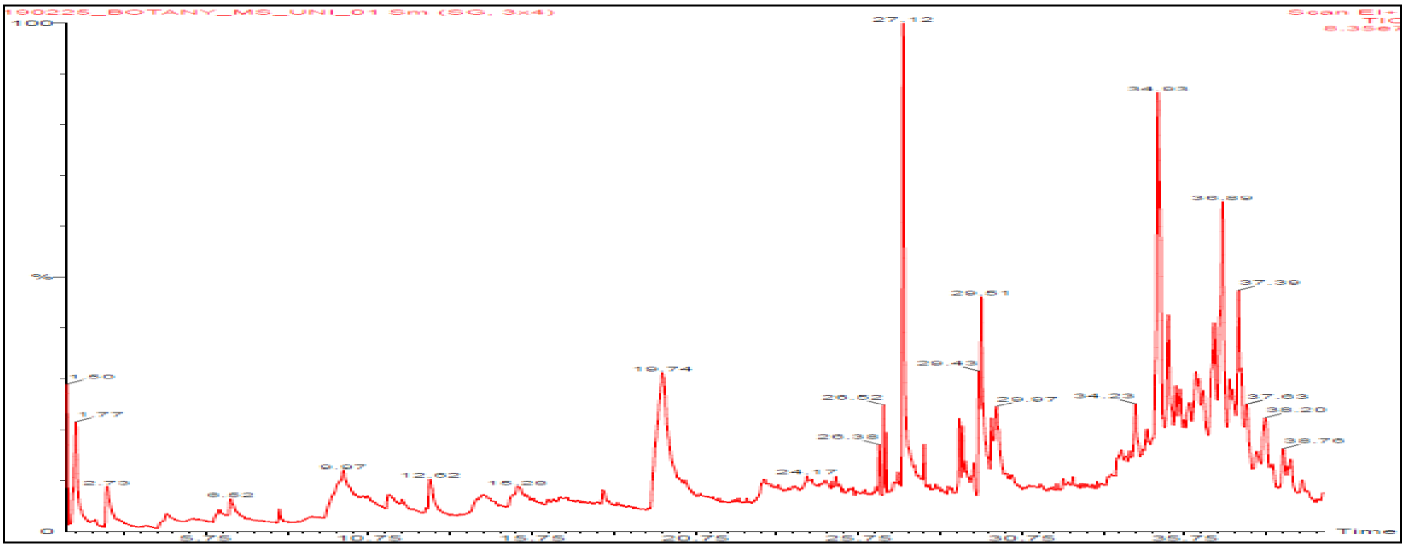

Fig 1 GC Chromatogram (Scan EI + TIC 8.35e7).

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Table 1 Retention Time (RT) and % Area for GC Chromatogram

Sr. No	RT	Components Name	Area	% Area
1	1.773	Comp 1	1826063	3.595341686
2	2.729	Comp 2	1110787.375	2.187033061
3	9.967	Comp 3	4748749	9.349828146
4	12.618	Comp 4	709265.563	1.396475393
5	19.741	Comp 5	11550963	22.74273055
6	26.383	Comp 6	292971.844	0.576833266
7	26.518	Comp 7	488558.75	0.961924994
8	26.588	Comp 8	315550.094	0.621287659
9	27.119	Comp 9	5645930.5	11.11629187
10	28.839	Comp 10	411549.563	0.810301342
11	28.929	Comp 11	296825.688	0.584421112
12	29.435	Comp 12	694242.563	1.366896556
13	29.51	Comp 13	1923709	3.787597229
14	29.83	Comp 14	386384.25	0.760753271
15	29.97	Comp 15	1143340.125	2.251126282
16	34.232	Comp 16	825137.313	1.624615677
17	34.927	Comp 17	6869837	13.52604556
18	35.237	Comp 18	1886363	3.714066562
19	36.642	Comp 19	2156462.5	4.245866391
20	36.893	Comp 20	4312337	8.490575067
21	37.393	Comp 21	2037115.5	4.010883675
22	38.203	Comp 22	1157550.25	2.279104646

Trifluoromethyl t-butyl disulfide (Compound 5) was detected at 19.741 minutes (MW: 190 g/mol, $C_5H_9F_3S_2$). This sulfur-containing compound may contribute to the plant's defense mechanisms or exhibit antimicrobial properties, as organosulfur compounds are often associated with biological activity. A notable high-molecular-weight compound, L-(+)-ascorbic acid 2,6-dihexadecanoate (Compound 9), eluted at 27.119 minutes (MW: 652 g/mol, $C_{38}H_{68}O_8$). This fatty acid ester of ascorbic acid (vitamin C) suggests potential antioxidant properties, possibly enhancing the extract's stability or therapeutic effects. The presence of bromoacetic acid, 2-ethylhexyl ester (Compound 17) at 34.927 minutes (MW: 250 g/mol, $C_{10}H_{19}O_2Br$) indicates halogenated organic constituents, which could arise from secondary metabolism or environmental interactions. Such compounds may possess bioactivity, though further toxicological assessment is needed. Finally, 2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)- (Compound 20), detected at 36.893 minutes (MW: 410 g/mol, $C_{30}H_{50}$), is structurally analogous to squalene, a triterpene precursor. This suggests the plant's capacity to synthesize terpenoid compounds, which are often linked to anti-inflammatory, antioxidant, or anticancer effects (Michel et al., 2016).

The diversity of these compounds underscores the phytochemical complexity of the plant mixture, warranting further investigation into their biological roles and potential medicinal applications.

IV. CONCLUSION

The GC-MS analysis of the polyherbal methanolic extract revealed 22 distinct phytochemical peaks, with four major compounds identified: trifluoromethyl t-butyl disulfide (antimicrobial potential), L-(+)-ascorbic acid 2,6-dihexadecanoate (antioxidant properties), bromoacetic acid, 2-ethylhexyl ester (bioactive halogenated compound), and a squalene-like terpenoid (anti-inflammatory/anticancer potential). The high abundance of these compounds, particularly Compound 5 (22.74% peak area), highlights their significance in the extract's medicinal properties, including antimicrobial, antioxidant, and anti-diabetic effects.

These findings validate the therapeutic potential of the polyherbal mixture Batrisu vasanu but also warrants further pharmacological and clinical studies to confirm efficacy and safety for potential therapeutic applications.

Table 2 Phytochemical Identification using m/z

Comp Name	RT	Mole. Weight	Chemical Name	Formula
Comp 5	19.741	190	Trifluoromethyl t-butyl disulfide	$C_5H_9F_3S_2$
Comp 9	27.119	652	L-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$
Comp 17	34.927	250	Bromoacetic acid, 2-ethylhexyl ester	$C_{10}H_{19}O_2Br$
Comp 20	36.893	410	2,6,10,14,18,22-tetracosahexaene,-2,6,10,15,19,23 hexamethyl-, (All-E)-	$C_{30}H_{50}$

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