RESEARCH PAPER

Scientific Validation of Sida Cordifolia oil Through Gc–Ms Analysis: Identification of Bioactive Compounds Relevant To Physiology of Labour And Facilitating Birth

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ABSTRACT

Introduction: Sida cordifolia oil (Bala Taila) is a traditional herbal oil formulation explained in Ayurveda classics. It is a medicinal oil used for pain relief, prenatal care, and Vata-related conditions. By controlling Apana Vata and facilitating normal labor (sukha Prasava), Ayurveda suggests utilization of Bala oil in various procedures to facilitate labor, especially during the ninth month of pregnancy. The phytochemical profile of Bala Taila and its potential involvement in labor modulation have not received enough attention, despite its widespread traditional use. Using Gas Chromatography–Mass Spectrometry (GC-MS) research, the current study required to discover the phytochemical components of Bala Taila and link the detected chemicals with possible pharmacological activities related to labour and pain management.

Materials & Methods: Bala Taila was purchased from a pharmacy with GMP certification and produced using traditional Ayurvedic methods as described in classical Ayurvedic text. At IIT Madras, an Agilent 8890 GC machine and an Agilent 5977 MSD were used to analyse ethyl acetate extract of the oil using GC-MS.

Result: Nineteen phytochemical elements were found in the investigation, including phenolic compounds, fatty acid derivatives, terpenoid esters, and phytosterols. β-sitosterol propionate had the largest peak area percentage (49.94%) among the chemicals found, followed by related sterol derivatives and campesterol acetate.

Discussion: Numerous substances, including derivatives of β-sitosterol, campesterol, and α-terpinyl acetate, have been found to have analgesic, anti-inflammatory, smooth muscle modulatory, and hormone-regulating properties. The traditional use of Bala Taila to support uterine physiology and reduce labor pain may be influenced by these pharmacological characteristics. The preponderance of sterol molecules points to a potential role in endocrine modulation and uterine smooth muscle function.

Conclusion: The study offers preliminary scientific proof in favor of Bala Taila's traditional therapeutic claims. To determine the precise mechanisms, target pathways, and effectiveness of each phytochemical implicated in labor promotion and pain management, more in-silico, experimental, and clinical research is required.

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INTRODUCTION

Evidence-based validation of classical Ayurvedic formulations is essential to bring traditional therapies into clinical practice. A series of events that take place in the genital organs to expel the viable products of conception out of the womb through the vagina is known as labour. Normal labour (Eutocia) is described as spontaneous in onset and at term (after completion of 37 weeks of gestation), with vertex presentation, without undue prolongation, and it should deliver naturally through the vagina with minimal aids. [1]

In Ayurveda normal labor is accomplished by regulated *Apana Vata* which is considered as regulating factor helps in effective uterine contractions and expulsion of fetus. [2] Classics explain a month-wise Antenatal Regime (*Garbhini Paricharya*) for healthy progeny. Oleation enema (*Anuvasana Basti*) has been described to be administered in the ninth month of pregnancy to facilitate labor and to avoid intra- partum and post-partum complications. It is a procedure of introducing medicated oil through the rectal route so as to prepare the birth canal for normal labor.

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Sida cordifolia is a perennial subshrub in the family Malvaceae. Studies have been conducted to check the bioavailability of compounds in S. cordifolia. Also, its pharmacological activities demonstrate significant anti-inflammatory, analgesic, and antioxidant effects. Its anti-inflammatory action is due to inhibition of pro-inflammatory mediators such as prostaglandins and cytokines, along with modulation of cyclooxygenase and nitric oxide pathways [1,2]. Also, *Sida cordifolia oil (Bala Oil)* is referred to in classics for various pain-dominant conditions, mainly caused by vitiated *vata* dosha[3]. It is described as strength-promoting, helps in pacifying Vata dosha, nourishing, and also has anti-inflammatory activities.

Contemporary pharmacological investigations validate the traditional claims, identifying multiple bioactive constituents, including ephedrine alkaloids, flavonoids, phytosterols, and phenolic compounds, that contribute to its therapeutic profile. But, no further research has been conducted on the oil-based form of S. cordifolia to validate its action on the uterus during labour. This study mainly validates the action of *Bala* Oil, a classical herbal formulation primarily prepared with Sida cordifolia Linn. (*Bala*) as the main ingredient, along with Tinospora cordifolia (Guduchi), Pluchea lanceolata (Rasna), and Rubia cordifolia (Manjishta), with Sesame oil as a base prepared with the standard method specified in classics [4]. GC-MS analysis of *Bala* Oil, done using Ethyl Acetate, is crucial for identifying a wide spectrum of volatile and semi-volatile phytochemicals based on solvent polarity, Ethyl Acetate separates semi-polar phenolic constituents.

MATERIALS AND METHODS:

Ethical statement:
Since the study doesn't have involvement of human or animal participants the study doesn't require Institutional Ethical committee or Institutional Animal Ethical Committee permission.
Study Design-

To analyze the phytoconstituents of *Bala Oil,* Gas Chromatography- Mass Spectrometry (GC-MS) analysis was performed using solvent, Ethyl Acetate. The Study was carried out at laboratory of Indian Institute of Technology, Madras.

Materials:

Formulation Details:
The main ingredient, *Bala Oil* was procured from the GMP Certified Pharmacy, Kerala Ayurveda which was prepared with classical method as mentioned in *Ashtang Hridaya*, *Chikitsa Sthana*, 21 chapter. After procuring *taila*, Qualitative (Test for specific Micro- Organisms) & Quantitative (Microbial limit test) was done to ensure safety and efficacy of the procured drug.
Chemical and Solvents:
GC- MS grade solvents-
Ethyl acetate
Analytical grade reagents for sample preparation.

Instrument-
GC-MS was performed with an Agilent 8890 GC system coupled with an Agilent 5977 MSD (Agilent: GC: 8890. GC/MS: Agilent 5977 MSD)

Sample Preparation for GC-MS Analysis-
Separate measured portions of *Bala Oil* each containing 1ml of oil were extracted using solvents, Ethyl acetate Extract.

Methods:

Extraction Procedure:

One hundred microliter sample of *Bala Oil* dissolved in 1 ml of each solvent The mixture was shaken rapidly to ensure thorough mixing of oil and solvents for 2 minutes. Sample were placed in an ultrasonic bath where high frequency sound waves were used to shake and agitate the mixture for 15 minutes. The upper clear layer was collected and filtered through a 0.22 um PTFE syringe filter. Filtered extracts were stored in GC vials at 4^{o} C till analysis.
Chromatography and Mass Spectrometry Method:
GC- MS analysis was performed using an Agilent 8890 GC system coupled with an Agilent 5977 MSD. An Agilent 8890 GC System combined with a 5977B GC/MSD System (single quadrupole) was used for the GC-MS Analysis at SAIF, IIT Madras, for screening phytoconstituents. The obtained results were analyzed using Version 2.3 - NIST MS Search (2017) was used for the library search. Electron Ionization was carried out with solvent –Ethyl Acetate of HPLC Grade. For all the Electron Ionization methods, 1 g of the medicated oil was extracted using 10 ml of the respective solvents and 1 µL extract was taken for analysis with the model in HP-5 MS Capillary Column. These conditions were maintained for the mentioned solvent; temperature range from -60 °C till 325 °C (350 °C) with a pressure of 11.367 psi and 40.402 cm/sec as the average velocity with the flow at 1.2 mL/min and dimensions of 30 m x 250 µm x 0.25 µm (Uncalibrated). The acquisition method was executed with 70 eV for the fixed electron energy with a quad temperature of 150 °C.

Result & Discussion:

The GC-MS analysis of Bala oil shown lipid-rich composition of the Bala oil along with a sterol-dominant composition such as campesterol derivatives, β-sitosterol propionate, stigmastane analogues. And has lipid rich composition such as unsaturated fatty acid esters, and selected aromatic and heterocyclic constituents. For normal labor various factors are essential like myometrial activation pathways, and coordinated endocrine, inflammatory pathways. The progress from uterine quiescence to active labour requires withdrawal of progesterone, estrogen predominance, upregulation of oxytocin receptors, and increased prostaglandin production [5,6]. The study identified phytosterols like β-sitosterol and campesterol. These compounds are structurally similar to cholesterol, which is the precursor of steroid hormones.

Sterols play an important role in maintaining membrane structure and stability and facilitate intracellular signalling pathways [7,8]. Estrogen increases uterine sensitivity in term pregnancy. Hence, sterols may contribute to receptor regulation and support normal contractile activity [9]. Unsaturated fatty acid derivatives such as 9-octadecenoic acid esters detected in the oil support a lipid-mediated mechanism. Fatty acids are essential for eicosanoid synthesis, and prostaglandins produced through lipid metabolism act as key regulators of cervical ripening and coordinated myometrial contractions.[10]. Alterations in membrane lipid composition influence prostaglandin availability and inflammatory signalling cascades that characterise normal parturition [11]. Thus, the combined presence of sterols and unsaturated lipid fractions provides a coherent biochemical framework consistent with the modulation of prostaglandin- dependent pathways essential for labour progression. A noteworthy finding in the present analysis is the identification of 1,3-di-tert-butylbenzene, a lipophilic substituted aromatic hydrocarbon. Although its direct role in reproductive physiology is not well characterised, substituted aromatic hydrocarbons are recognised for their capacity to interact with hydrophobic domains of membrane proteins and lipid bilayers.

The *Bala taila* has the main 4 ingredients i.e. *Sida cordifolia (Bala), Tinospora cordifolia* (*Guduchi)*, *Pluchea lanceolata* or *Alpinia galanga* (*Rasna)* and Sesame oil (*Tila taila)* among this all the 4 *dravyas* have its activity that help in reducing the *Vataja* disorders i.e. related to neurological pains. [12] The ingredients of *Bala taila* have actions mainly related to pain related disorders and the traditional use of *Tila Taila*, the presence of phytosterols, and the experimentally shown analgesic and anti-inflammatory properties of *Guduchi* may all work together to modulate pain related to uterine contractility and helps to reduce labor complications.

Based on the *Ayurvedic* pharmacological principles i.e. *Rasa Panchaka*, the oil works mainly on few principles such as decreasing the obstructed *Vata* especially *Apana vata* (the one which concentrates mainly on the excretory and reproductive system). Henceforth, with this we know that *Bala oil* has its action on the system. Although the *Ayurvedic* pharmacology is a wider one, we need the phytochemicals that work specifically on the labor pain, thus with the GCMS we can find out the activity of the oil in various diseases.

Here the solvent mainly used was Ethyl acetate, it was used as the extraction solvent for GC-MS analysis because it could dissolve a wide range of semi-polar and non-polar phytoconstituents found in the medicinal oil. [13] In the result we got a total of 19 phytochemicals among this, β-Sitosterol had the highest area coverage with having a strong presence in the *taila*, this may be due to the reported involvement of phytosterols in uterine smooth muscle modulation and steroid-related physiological pathways. Although the phytochemical directly doesn't have any clinical relevance on labour pain, it may contribute to the hormonal modulation and smooth muscle relaxation. Since comparable sterol compounds have previously shown effects on uterine contractility in pharmacological experiments, the presence of phytosterols like β-sitosterol and campesterol derivatives may contribute to uterine smooth muscle regulation and possibly uterotonic activity. [14]

When β-sitosterol or its derivatives are detected in GC-MS of oils, it indicates
the presence of a lipid fraction rich in sterols, the composition of mature fatty oils, nutraceutical potential, and potential endocrine or anti-inflammatory bioactivity. Phytosterols, terpenoid esters, and fatty acid derivatives such α-terpinyl acetate, β-sitosterol derivatives, and campesterol derivatives were found in the GC-MS profile. These compounds are said to have analgesic and anti-inflammatory properties. Together, these ingredients might increase the formulation's capacity to reduce pain.[15] The identified phytoconstituents may contribute to labour-associated supportive activities, such as smooth muscle modulatory, analgesic, and anti-inflammatory effects, which could aid in the management of labour discomfort.

Among the phytochemicals obtained most of the chemicals have one or the other activity related to the labour pain hence we may suggest that *Bala taila* has activity on labour pain, and moreover the mode of administration i.e. According to Acharya *Charka*, *Vayu* is most likely to be in a vitiated state during pregnancy. *Anuvasana basti* (oil enema), counteracts the all the properties that are predominant in labour pain. In addition to these *Basti* of *taila*, it also does *manaprasada*, *veerya*, *bala*, *varna*, and *agnipushti*. It is risk-free and cures all illnesses and regulates normal functions. According to *Charaka* claims that when given *Anuvasana basti* nourishes all channels once it reaches the *nabhi* (main seat of Sira and Dhamni). [16]

Although the phytochemicals obtained in the study have the action on labour pain, the study still lacks which is the exact phytochemical among the 19 and the study could have improved if other solvents such as hexane and chloroform was used to perform the study. Furthermore in-silico study can be performed with the phytochemicals to establish the activity of the *Bala oil* in the labour pain with making the target that is involved in the pain. Moreover, clinical study should be performed so as to suggest that *Bala oil* has its effect on labour promotion in a smooth manner.

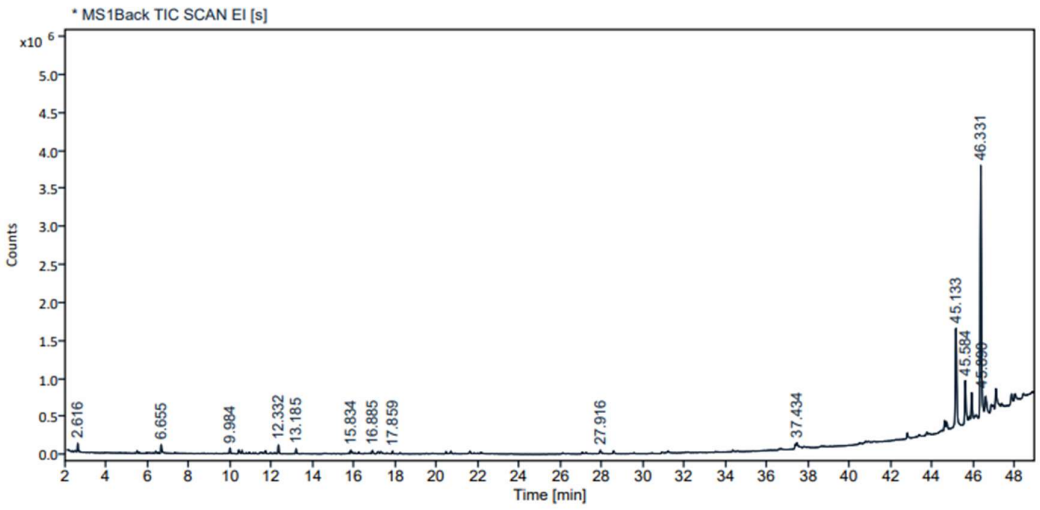

Figure.2. Area and Peak of the *Bala oil* GCMS

Table.1. Phytochemicals of GCMS analysis

Sr. No.	Compound Name	Retention time	Area %	Prob%
	3-Furanmethanol	2.616	1.12	63.35
	2-Furanmethanol	2.616	1.12	15.9
	Maltol	6.655	1.88	64.1
	3-Acetoxy-2-methyl-pyran-4-one	6.655	1.88	18.16
	Benzene, 1,3-bis(1,1-dimethylethyl)-	9.984	0.81	36.94
	7-Methoxy-2-methylquinolin-4-ol	9.984	0.81	20.16
	α-Terpinyl acetate	12.332	1.36	17.99
	Hexanoic acid, hexyl ester	13.185	0.76	51.35
	Tetradecane, 2,6,10-trimethyl-	15.834	0.42	15.92
	Hexanoic acid, octyl ester	17.859	1.22	28.88
	Azuleno[4,5-b]furan-2(3H)-one, decahydro-3,6,9-tris(methylene)-, [3aS-(3aα,6aα,9aα,9bβ	27.916	0.64	83.28
	9-Octadecenoic acid (Z)-, oxiranylmethyl ester	37.434	1.63	42.37
	Campesterol, acetate	45.133	23.7	46.06
	Campesterol, propionate	45.133	23.7	32.52
	Stigmasta-5,22-dien-3-ol, acetate, (3β,22Z)-	45.584	9.74	28.45
	Stigmastan-3,5,22-trien	45.584	9.74	18.95
	Stigmast-5-en-3-ol, oleate	45.89	6.3	17.35
	β-Sitosterol, propionate	46.331	49.94	47.78
	Stigmasta-3,5-diene	46.331	49.94	21.76

Conclusion:

Ayurveda has multiple polyherbal formulations that work on a synergistic manner, here it doesn't work on single principle or a pathway. One such polyherbal combination is *Bala oil*, although the oil is indicated in many diseases, the phytochemicals that are present in the formulation was still unclear. Research is done so as to fulfill the unknown gaps, to accomplish the gap this research was done. *Bala oil* although known for its analgesic and anti-inflammatory action, still the classically indicated *Apana vatahara* action is unclear because of the unexplored of the phytochemicals and their action.

The result of the Gas chromatography had shown the major presence of sterols this may be due to the oil content that is present in the *Bala oil*. The sterols not only help in the smooth muscle contraction of the uterus but also it helps in the analgesic activity that is needed for labor process. With GCMS, the phytochemicals present in the oil is known but which phytochemicals is having the major action on the disease or the labor process can be known with the help of in-silico analysis, followed by that validation of the ancient wisdom can be achieved by performing a clinical trial.

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