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The leaf of *Vitex negundo* Linn. is indicated in pain dominant *vata* diseases. The leaf processed with sesame oil and leaf in water media (as decoction) is used in different procedures to manage painful *vata* conditions. This study has revealed the chemical composition of sesame oil, medicated oil and *kashaya* (decoction) of *V. negundo* and can serve as standard reference for identification and initial step for further studies like absorption study and so on.

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Short Communication

GCMS Study of *Taila* (medicated oil) and *Kashaya* (decoction) *Kalpana* of *Nirgundi* (*Vitex negundo* Linn.)

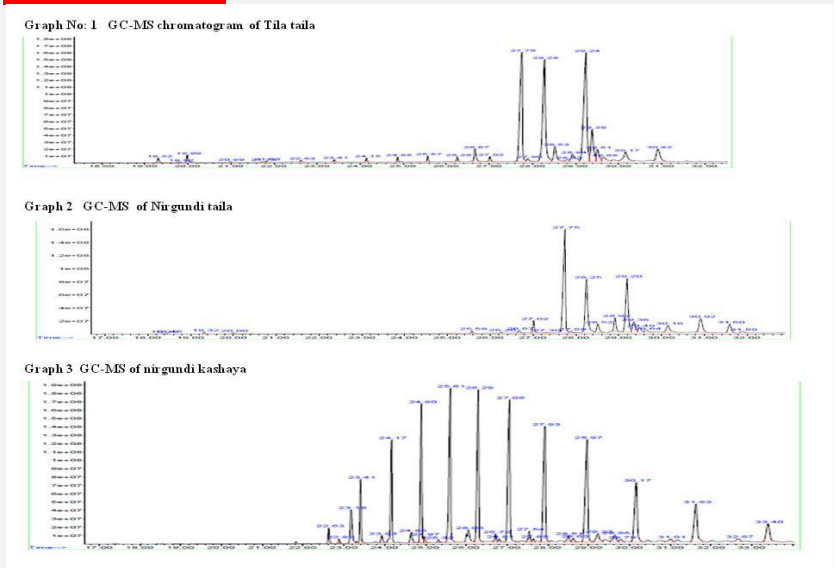
Padmakiran Chinivar*, Prasad Uchangi, Sunil Kumar Koppala Narayana[1]
Department of Panchakarma, Sri Dharmasthala Manjunatheshwara College of Ayurveda, Kuthpady, Udupi 574118. [1]Department of Pharmacognosy, Siddha Central Research Institute, Arumbakkam, Chennai 600106, India.

ABSTRACT

Introduction: Sesame oil is the base for preparation of most of the medicated oils used in *Ayurvedic* treatments. The oil is beneficial in the management of *vata* diseases. The leaf of *Vitex negundo* Linn. is indicated in pain dominant *vata* diseases. The leaf processed with sesame oil and leaf in water media (as decoction) is used in different procedures to manage painful *vata* conditions. ***Methods:*** Gas chromatography coupled with mass spectroscopy (GCMS) has been used for detection and identification of volatile components from the oil of *Sesamum indicum* DC of Pedaliaceae, medicated oil and decoction from the leaf of *V. negundo* of Verbenaceae. ***Results:*** 26 compounds were identified in *Tilataila* (Sesame oil), 22 compounds were identified in *Nirgunditaila* (medicated oil prepared from *V. negundo*) and 30 compounds were identified in *Nirgundikashaya* (decoction of *V. negundo*). Comparatively 9 compounds identified were common to both *Tilataila* and *Nirgunditaila*. Only one compound Hentriacontane was found to be common with *Nirgunditaila* and *Nirgundikashaya*. ***Conclusion:*** This study has revealed the chemical composition of sesame oil, medicated oil and *kashaya* (decoction) of *V. negundo* and can serve as standard reference for identification and initial step for further studies like absorption study and so on.

KEYWORDS Decoction, *Nirgunditaila*, *Nirgundikashaya*, *Tilataila*, *Vata* disease.

PICTORIAL ABSTRACT



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CORRESPONDENCE Dr. C Padmakiran, Associate professor, PG Department of Panchakarma, SDM college of Ayurveda, Kuthpady, Udupi 574118, India. Email: kiranasdm@gmail.com.

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The quality medicine used in the treatment is one of the essential contributory factors for the success of the treatment.[1] Among the different form of medicines used in the Ayurvedic treatments, the oils and the decoctions are the essential parts in the management of different diseases. *Tilataila* (Sesame oil) is considered to be best[2] and it is used as the base for preparation of most of the medicated oils used in Ayurvedic treatments. Ayurvedic texts identified the difference in action by the same drug when they are used in different formulations.[3] Hence with this in backdrop, a single drug as leaf of *Vitex negundo* Linn. of Verbenaceae is prepared in oil media as *Nirgunditaila* (with sesame oil as a base) and in water media as *Nirgundikashaya* is selected. An attempt has been made to find out chemical composition of all the three forms of medicine through the Gas chromatography-Mass spectrometry (GC-MS). Another aim was to compare the same drug (*Nirgundi*) when prepared in different forms.

1. Plant collection and formulation

Sesame oil is procured from the local oil mill. The *Nirgundi* leaf is collected from the herbal garden of SDM College of Ayurveda, Udupi and the authentication of the leaf was done at Dravyaguna department of the college. *Nirgundi* oil is prepared in the laboratory of the Bhaishajya kalpana Department. *Nirgundi* decoction is prepared in the Panchakarma unit of the hospital.

2. Method of preparation of Nirgunditaila

An Iron vessel was taken with equal quantity of *Tilataila* and *Nirgundi patra swarasa* (fresh juice of *Nirgundi* leaf) and four times of water was added and kept for boiling.[4,5] Heating

continued with continuous stirring. The procedure of boiling is continued for 2 days.[6] Then on the third day mixture was boiled till *sneha siddhi lakshanas* (Sign of proper prepared oil) were observed. Then oil was filtered through a clean white thick in its warm stage and stored in an air tight container.

3. Method of preparation of Nirgundikashaya

Nirgundi fresh leaves were collected and washed thoroughly. They are chopped into small pieces. In a vessel, chopped *nirgundi* leaves are taken and 4 parts of water is added to it and boiled till the boiling point is reached. Then the lid is closed and *kashaya* is kept as it is for some time. The decoction is then filtered using clean white thick cloth and used for analysis.[7]

4. Sample preparation

Tilataila and *Nirgunditaila* – The unsaponifiable content of the oils were dissolved in *n*-hexane. *Nirgundikashaya* – *n*-Hexane soluble portion of the *kashaya* was separated.

5. Gas chromatography coupled with mass spectroscopy (GCMS)

This is the method of choice for the separation of volatile substances or the volatile derivatives of certain non-volatile substances.[8] The identification of the unknown compounds is achieved by comparison of their full mass spectrum with a mass spectral library or database. The main limitation of GC-MS is, the compounds must be sufficiently volatile to allow transfer from the solid phase to the mobile carrier gas and thus elute from the analytical column to the detector. Larger numbers of compound are still too polar or too large in size to be analyzed with this technique.

Instrument - GC-MS-5975C [AGILENT]. Column Oven Temperature - 70˚C; Injector Temperature - 250˚C; Injection Mode – Split; Split Ratio – 10; Flow Control Mode - Linear Velocity; Column Flow - 1.5 ml/min; Carrier Gas - Helium 99.9995% purity; Injection volume - 1 microlitre; COLUMN - DB-5ms Agilent; Length - 30.0m; Diameter - 0.25mm; Film Thickness - 0.25um.

Column Oven Temperature Program

Rate	Temperature(˚C)	Hold Time(min)
-	70.0	3.0
10	300	9.0 [35.0 mts total]

Ion source temp - 230 °C; Interface temp - 240°C; Scan range - 40–700 m/z; Solvent cut time - 3 mins; MS start time - 3(min); MS end time - 35(min); Ionization - EI (-70ev); Scan speed – 2000; MS LIBRARY NIST- 11

GC-MS of *tilataila* (TT) revealed presence of 26 constituents, 13 of them could not be identified. .gamma.-Sitosterol (25.72%) and 1,3-Benzodioxole, 5,5'-(tetrahydro - 1H,3H-furo[3,4-c]furan-1,4-diyl) bis, [1S-(1.alpha.,3a.alpha.,4.beta.,6a.alpha.)]- (21.36%) were the major constituent identified in the sample. (Table 1 and Graph 1). *Nirgunditaila* (NT) has revealed presence of 22 constituents out of them 11 could not be identified. 1,3-Benzodioxole, 5,5'-(tetrahydro - H,3H-furo[3,4-c]furan-1,4-diyl) bis-, [1S-(1.alpha., 3a.alpha., 4.beta., 6a.alpha.)]- (30%) is major compound identified (Table 2 and Graph 2). *Nirgundikashaya* (NK) showed presence of 30 constituents out of which 11 could not be identified. Nonadecane (12.24%), Tricosane (11.20%), 11-Tricosene (10.76%) and 1-Octadecene (10.54%) are the major constituents identified from NK (Table 3 and Graph 3).

Comparatively 9 compounds identified were common to both TT and NT. As NT is prepared using TT as base there are every chance common constituents in both. NK is a water based drug prepared using *Nirgundi* leaf (NL), and hence it found to have comparatively different chemical composition. Only one compound Hentriacontane was found to be common with NT and NK which is obviously due to addition of NL to both the preparation though in different base. Extraction of NL in oil base and aqueous base rendered completely different chemistry between the two (Table 4).

Table 1. Compounds detected by GC-MS of Tilataila (base oil)

Peak	RT	Area %	Name	SI
1	19.322	0.62	Phytol	99
2	19.816	0.19	Unidentified	-
3	19.990	0.96	trans-Geranylgeraniol	99
4	20.993	0.08	Unidentified	-
5	21.733	0.09	Unidentified	-
6	21.820	0.18	Unidentified	-

7	22.634	0.27	Unidentified	-
8	23.403	0.28	Unidentified	-
9	24.159	0.41	Unidentified	-
10	24.870	0.46	Unidentified	-
11	25.567	0.57	Unidentified	-
12	26.264	0.47	Unidentified	-
13	26.671	1.96	gmma.-Tocopherol	98
14	27.020	0.70	Eicosane	98
15	27.760	21.36	1,3-Benzodioxole, 5,5'-(tetrahydro -1H,3H furo[3,4-c]furan-1,4-diyl)b is-, [1S (1.alpha.,3a.alpha.,4.beta.,6a.alpha.)]-	96
16	27.905	0.56	Unidentified	-
17	28.283	22.00	Unidentified	-
18	28.530	3.28	Stigmasterol	99
19	28.820	0.52	Unidentified	-
20	28.937	2.31	Obtusifoliol	95
21	29.242	25.72	gamma.-Sitosterol	99
22	29.387	6.40	Stigmasta-5,24(28)-dien-3-ol,(3.beta.,24Z)-	91
25	30.171	2.98	9, 19-Cyclolanost-24-en-3-ol, (3.beta.)-	99
26	30.926	3.54	Pregn-17(20)-en-16-one, (5.alpha., 17Z)-	83

Table 2. Compounds detected by GC-MS of *Nirgunditaila*

Peak	RT	Area %	Name	SI
1	18.349	0.16	Unidentified	-
2	18.451	0.19	Unidentified	-
3	19.322	0.52	Phytol	91
4	19.990	0.47	trans-Geranylgeraniol	99
5	25.582	0.41	Eicosane	99
6	26.264	0.17	Unidentified	-
7	26.671	0.78	gamma.-Tocopherol	95
8	27.020	2.85	Hentriacontane	98
9	27.296	0.31	Unidentified	-
10	27.746	30.00	1,3-Benzodioxole, 5,5' (tetrahydro -1H,3H-furo[3,4c]furan-1,4-diyl)b is- , [1S-(1.alpha.,3a.alpha.,4.beta., 6a.alpha.)]-	96
11	27.891	0.60	Unidentified	-
12	28.254	18.48	Unidentified	-
13	28.515	2.95	Stigmasterol	99

14	28.922	4.18	Unidentified	-
15	29.198	17.95	gamma.-Sitosterol	99
16	29.358	3.79	Fucosterol	87
17	29.489	1.80	Unidentified	-
18	29.648	0.99	Unidentified	-
19	30.157	3.33	9, 19-Cyclolanost-24-en-3-ol, (3.beta.)-	99
20	30.926	5.86	9, 19-Cyclolanostan-3-ol, 24-methylene-, (3.beta.)-	95
21	31.594	3.47	Unidentified	-
22	31.899	0.75	Unidentified	-

Table 3. Compounds detected by GC-MS of *Nirgundikashaya*

Peak	RT	Area %	Name	SI
1	22.634	0.83	Nonadecane, 9-methyl-	95
2	22.881	0.22	Phthalic acid, di(2-propylpentyl) ester	87
3	23.186	2.75	Unidentified	-
4	23.418	3.09	Hexacosane	98
5	23.941	0.56	Unidentified	-
6	24.173	6.14	Unidentified	-
7	24.652	0.89	Unidentified	-
8	24.899	7.86	Octacosane	91
9	24.972	0.48	Unidentified	-
10	25.321	0.18	Heneicosane	97
11	25.611	11.20	Tricosane	94
12	26.061	1.48	Unidentified	-
13	26.294	10.76	11-Tricosene	95
14	26.715	0.54	Triacontane	97
15	26.802	0.22	Docosane, 9-octyl-	91
16	27.063	12.24	Nonadecane	96
17	27.543	0.81	Hentriacontane	95
18	27.644	0.25	Unidentified	-
19	27.935	10.54	1-Octadecene	92
20	28.501	0.63	Unidentified	-
21	28.632	0.36	Hexadecane, 1-iodo-	90
22	28.966	10.58	Unidentified	-
23	29.227	1.14	4H-1-Benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-5-hydroxy-3,6,7-trimethoxy-	95
24	29.648	0.91	Heptadecane, 9-octyl-	93
25	29.794	0.34	Hexadecane, 1-iodo-	93
26	30.171	6.80	Heneicosane, 11-pentyl-	94
27	31.014	0.49	Unidentified	-
28	31.638	4.90	Nonadecane, 9-methyl-	93
29	32.669	0.48	Unidentified	-
30	33.395	2.33	Tetracosane	97

Table 4. Comparison of compounds identified by GC-MS of Tilataila (base oil), *Nirgunditaila* and *Nirgundikashaya*

Tilataila (base oil)	*Nirgunditaila*	*Nirgundikashaya*
Phytol	Phytol	
trans-Geranylgeraniol	trans-Geranylgeraniol	
Eicosane	Eicosane	
gmma.-Tocopherol	gamma.-Tocopherol	
--	--	Nonadecane, 9-methyl-
--	--	Phthalic acid, di(2-propylpentyl) ester
--	--	Hexacosane
--	--	Octacosane
--	--	Heneicosane
--	--	Tricosane
--	--	11-Tricosene
--	--	Triacontane
--	--	Docosane, 9-octyl-
--	--	Nonadecane, 9-methyl-
--	--	Nonadecane
--	Hentriacontane	Hentriacontane
--	--	1-Octadecene
--	--	Hexadecane, 1-iodo-
--	--	4H-1-Benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-5-hydroxy-3,6,7-trimethoxy-
--	--	Heptadecane, 9-octyl-
--	--	Hexadecane, 1-iodo-
--	--	Heneicosane, 11-pentyl-
--	--	Nonadecane, 9-methyl-
--	--	Tetracosane
1,3-Benzodioxole, 5,5'-(tetrahydro -1H,3H-furo[3,4-c]furan-1,4-diyl)bis-, [1S-(1.alpha.,3a.alpha.,4.beta.,6a.alpha.)]-	1,3-Benzodioxole, 5,5'-(tetrahydro -1H,3H-furo[3,4-c]furan-1,4-diyl)bis-, [1S-(1.alpha.,3a.alpha.,4.beta.,6a.alpha.)]-	--
Stigmasterol	Stigmasterol	--
Obtusifoliol	--	--
gamma.-Sitosterol	gamma.-Sitosterol	--
--	Fucosterol	--
Stigmasta-5,24(28)-dien-3-ol, (3.beta.,24Z)-	--	--
9,19-Cyclolanost-24-en-3-ol, (3.beta.)-	9, 19-Cyclolanost-24-en-3-ol, (3.beta.)-	--
Pregn-17(20)-en-16-one, (5.alpha., 17Z)-	--	--
--	9, 19-Cyclolanostan-3-ol, 24-methylene-, (3.beta.)-	--

The Gas chromatography-Mass spectrometry is the method of choice for the separation of volatile substances or the volatile derivatives of certain non-volatile substances. GCMS of *Tilataila*, *Nirgunditaila* and *Nirgundikashaya* identified 26 compounds, 11 compounds and 30 constituents respectively. Different compounds are identified in oil and decoction form. The identified composition can be used for standardization of these drugs. This can also be used as the first step for further

studies like absorption studies. The composition identified here can be compared with the GCMS of the sample collected during the absorption studies.

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Conflict of interest None

Contributors Dr Padmakiran and Dr Prasad conceptualized the study and prepared article on the findings. Dr KN Sunil Kumar planned and executed the study, interpreted the results and made the write up suitable for publication.

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