Research Article

Gas Chromatography - Mass Spectrometry (GC-MS) Profiling and Phytochemical Characterization of Bilva Taila A Traditional Formulation For Badhirya

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ABSTRACT

Background: Badhirya is one among the 28 Karnagata rogas. As per WHO, by 2050 one in every ten people will be affected by hearing loss. This itself shows the importance of treating hearing loss. As per Ayurveda Badhirya is of two types Vataja and VataKaphaja. The basic line of management includes Snehana and Rasayana. Karnapurana (instillation of medicine into external auditory canal) is a major treatment style adopted by the practitioners for treating Badhirya if tympanic membrane is intact. Bilvataila is widely used in practise and it is directly indicated for both types and successful results are available. Aim:To prepare Bilva taila, ensure its safety and identify the phytoconstituents present in it. Objective: To prepare Bilva taila according to Taila Kalpana and to analyse its Physico-chemical parameters, test for heavy metals and microbes for ensuring safety and purity of drug and also to conduct the Gas Chromatography Mass Spectrometry analysis of Bilvataila and thereafter analysing the compounds for their role in treating hearing loss. Materials and methods: GCMS analysis is carried out at Amrith Labs with instrument Shimadzu and identified compounds pharmacological action was collected from various other articles and textual references. Result and Discussion: The physiochemical analysis of the drug was comparable with API standards. Presence of heavy metals was as per permissible limits, no detectable microorganisms in microbiological testing GCMS analysis identified 50 compounds. Oleic acid butyl ester constituted an area of 56.91% followed by Octadecanoic acid ,3-hydroxy-2-tetradecyl methyl ester 17.68%. The identified compounds mainly act as anti-microbial, anti-inflammatory, antidegenerative, anti-oxidant and neuroprotective. Conclusion.All the analysis reports suggest that the drug is having API standard and is safe for use.

KEYWORDS Bilvataila, GCMS, Karnapurana, Badhirya, Conductive hearing loss, Sensorineural hearing loss.

INTRODUCTION

Bilva Taila is a classical ayurvedic medicated oil having reference in Bhaishajya Ratnavali Karnaroga Chikitsa Adhyaya.[1] It is prepared with Bilva phala, Gomutra, Ajaksheera and Tilataila as ingredients.It is specifically indicated in Badhirya (Hearing loss or reduced earing) in the form of Karnapurana (instillation of medicated drops to ear). Since years practitioners are using this formulation for hearing loss and other ear related diseases and is found to be effective.

As per WHO a person who is not able to hear as well as someone with hearing-hearing thresholds of 20db or better in both is said to have hearing loss. [2] Based on pathology it is of three types Conductive hearing loss, Sensorineural Hearing loss and Mixed hearing loss. In Conductive hearing loss the pathology lies from external ear to stapediovestibular joint. The main reason include wax, inflammatory swelling in EAC, middle ear, ASOM, CSOM tympanic membrane perforation, eustachian tube obstruction, otosclerosis etc. Sensorineural hearing loss (SNHL) results due to damage to the hair cells within the inner ear, the vestibulocochlear nerve or central processing centres of the brain. The aetiology may be congenital, traumatic, infectious, inflammatory, iatrogenic, neoplastic, senile or miscellaneous. [3]

As per Ayurveda, hearing loss can be considered as Badhirya. It arises due to vitiated Vata or Vatakapha or neglected Karnanada. Thus, it can be divided into two types- Vataja and Vatakaphaja.[4] The Lakshanas of Badhirya includes Kruchrashruti (hearing loud sound with difficulty) or loss of hearing. Among the various treatment method Karnapurana is usually practised for Karnarogas and Bilva Taila is exclusively a drug of choice in Badhirya. [5] For enhancing the scientific knowledge on how the drug works a GCMS analysis of this oil has been carried out.

OBJECTIVE

1.To access the organoleptic characters of Bilvataila and undergo physicochemical and GCMS Analysis.

2. To analyse the compounds in Bilva Taila obtained after GC-MS analysis and to interpret how it helps in treating Badhirya.

MATERIALS AND METHODS

Drug collection identification and authentication

Apakva Bilva Phala (Aegle marmelos) was collected and Murchita Tila Taila (Sesame oil) was procured from GMP certified KLE's Ayurveda Pharmacy at Khasbag, Belagavi Karnataka. Drug identification and authentication was done at Central Research Facility of Shri BMK Ayurveda Mahavidyalaya, Belagavi an AYUSH approved ASU Drug testing laboratory. Voucher specimen number for Bilva Phala Majja is CRF/Auth/267/2025-2026. Fresh Aja Ksheera(Goat milk) and Gomutra (cow's urine) was collected from local farmers of Belagavi.

Method of preparation.

Bilva Taila was prepared from GMP certified KLE's Ayurveda pharmacy in Khasbag, Belagavi. Table 1 shows the ingredients and proportion of each Dravya. Apakwa Bilva Phala Majja was collected from the fruit by separating the seeds and made into a Kalka(bolus) and kept aside. Required quantity of Tila Taila was measured and taken in a vessel it is heated over mild flame. This helps in evaporation of moisture after that Kalka is added and thoroughly mixed. Then Dravadravyas are added and subjected to mild heat, maintaining the temperature. Contents to be stirred continuously in order to avoid charring. Taila to be boiled till Kharapaka Lakshanas are attained which include passing wick test,where Kalka attains perfect wick shape when rolled, in Kharapaka Kalka will be rough to touch. Sound test absence of crackling sound when Kalka is subjected for heating over fire. Phenodgama (appearance of froth) and Gandha Varna Rasa Utpathi (attaining characteristic smell, colour and taste). [6] Later Taila was filtered through a clean cloth and stored in an air tight container after cooling.

Table I. ingredients of Bilva taila

Sl.no	Ingredients	Latin/English name	Parts used	Proportion
1	Bilva Phala Majja (Apakva)	Aegle Marmelos Corr.	Phala Majja	1 part
2	Aja Ksheera	Goat milk		16 parts
3	Go Mutra	Cows' urine		16 parts
4	Jala	Water		16 parts
5	Tila Taila	Sesamum Indicum Linn.	Seed	4 parts

Analysis

The test conducted includes Organoleptic evaluation, Physico chemical screening, test for specified microorganism (qualitative), microbial limit test (quantitative) and test for heavy metals. These tests were carried out in the AYUSH approved ASU Drug testing Laboratory of KAHER's Shri BMK Ayurveda Mahavidyalaya, Belagavi, Karnataka by following the standard procedures in accordance with Ayurvedic Pharmacopeia of India. Instruments used for specific analysis are listed below.

Halogen moisuture analyzer (Temp 40-200^{o}C) for accessing loss on drying. Saponification value by Reflux apparatus and titration with a standard burette. Abbe refractometer (1.300-1.700 Ri) at 40 ^{o}C for refractive index, Pycnometer (25-50mL) at 25 ^{o}C for specific gravity.

Test for heavy metals was done with atomic absorption Spectrophotometer, Model: PerkinElmer Analyst 400, Detection limit 0.001 -0.01 ppm. Test for microorganisms was done using sterile techniques in a laminar air flow cabinet, incubated in a temperature-controlled incubator (37 ^{o}C for bacteria, 25 ^{o}C for fungi) and observed under microscope.

GCMS Analysis

Bilva Taila was subjected to GCMS analysis at Amrith Labs Nisargram Pvt Ltd. Shimoga Karnataka. The instrument used was Shimadzu GC_MS QP-2010SE model.

For sample preparation 0.1ml of Taila was diluted with 10ml of petroleum ether the extract was filtered by 0.2µm nylon syringe filter of 13mm. 1µL of the solution were subjected to analysis.

DB-5MS capillary coloumn (30m x0.25mm internal diameter x 0.25µm film thickness) is used for performing chromatographic separation. The injector temperature was maintained at 280^{o}C and the sample was introduced in split mode with split ratio1:10. High purity Helium gas was used as the carrier gas with a coloumn flow rate of 1.50mL/min, linear velocity of 45.1cm/s and purge flow of 3.0mL/min.

The oven temperature is as follow

Rate (^{o}c/ min)	Temperature (^{o}c)	Hold time (min)
-	80	2
10	280	10

20 330 5

Electron impact (EI) ionization mode with an ionization energy of 70eV by maintaining the ion source temperature at 200°C and interface at 300°C is used to perform Mass spectrometry. The solvent cut time was fixed at 1.40 min to minimize solvent interference and the detector gain mode was adjusted relative to the tunng result with a gain of 0.95kv. The mass spectrometric acquisition parameters for group 1 event

Start time: 2min; scan speed :1666

End time :33min; start m/z:35.00

ACQ mode: Scan; End m/z: 500.00

Event time: 0.30 sec

These conditions are set and allowed for precise separation and identification of analyst. The compounds were identified by NIST 20 (National Institute of Standards and Technology) Library data base.

RESULT

Result of analysis are tabulated as organoleptic characters of prepared Bilva Taila Table 2, Physico-chemical characteristic, qualitative and quantitative test for micro-organism and test for heavy metals as table 2 to 5. Total quantity of Taila taken is 1 Litre obtained is 800ml and loss of Taila is 200ml

Table: 2 Organoleptic Characters of Bilva Taila

Sl.no	Parameters	Results
1	Colour	Orange
2	Odour	Mild Characteristic
3	Form	Taila

Table: 3 Physico chemical characteristics of Bilva tail

Sl.no	Tests	Results
1	Loss on drying at 110°C	6.910%
2	Refractive index at 40°C	1.480
3	Acid value	4.127
4	Iodine value	75.94
5	Saponification value	199.37
6	Specific gravity	0.91

Table: 4 Test for specified micro-organism (qualitative) and microbial limit test (quantitative)

Bacteria	Limits	Results
E. coli	Absent/100ml	Absent
S. aureus	Absent/100ml	Absent
S. abony	Absent/100ml	Absent
P. aeruginosa	Absent/100ml	Absent
Microbial limit test		
Total bacterial count	30-300cfu/ml*	No growth
Total fungal count	10-100cfu/ml	No growth

*cfu-colony forming units.

Table: 5 Test for heavy metals

Sl.no	parameter	Result	Permissible limits
1	Lead (Pb)	Less than 0.1ppm	10ppm
2	Arsenic (As)	Less than 0.1ppm	3ppm
3	Cadmium (cd)	Less than 0.1ppm	0.3ppm
4	Mercury (Hg)	Less than 0.1ppm	1ppm

*ppm-parts per million.

GC-MS Analysis of Bilva Taila identified 50 compounds.GC-MS profile of Bilva Taila is given in figure 1.The list of 50 compounds along with peak retention time, area, area percentage and height are given in figure 2. Some of the identified compounds has been tabulated for retention time area percentage and possible pharmacological action as table 7

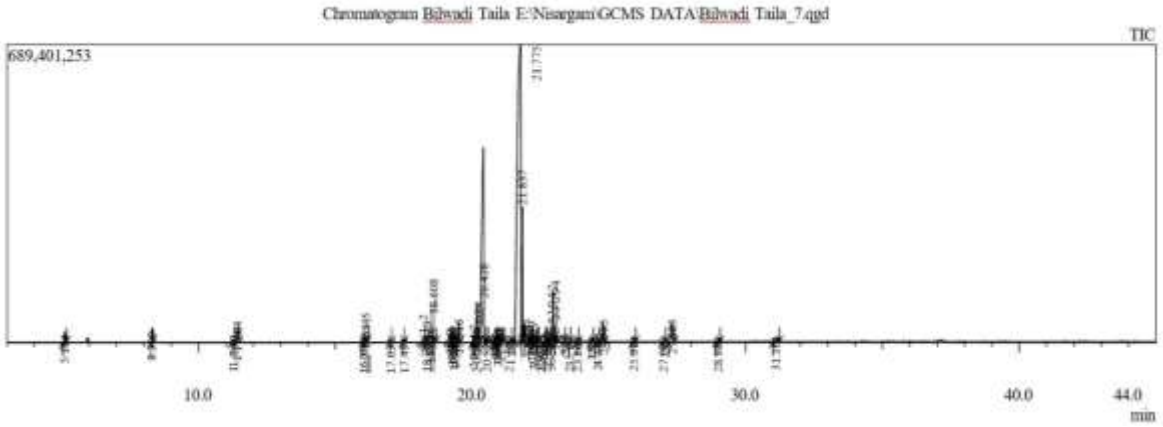


Figure 1 GCMS profile of Bilva taila (x axis -retention time and y axis-Total ion current)

Peak#	R.Time	Area	Area%	Height	CAS#	Name
1	5.114	53681653	0.45	23597638	106-70-7	Hexanoic acid, methyl ester
2	8.300	46351774	0.39	31188350	111-11-5	Octanoic acid, methyl ester
3	11.270	8086571	0.07	3104772	7367-83-1	4-Decenoic acid, methyl ester, Z-
4	11.434	97290488	0.82	34993498	110-42-9	Decanoic acid, methyl ester
5	16.028	3935863	0.03	1611114	56630-73-0	9,15-Octadecadienoic acid, methyl ester
6	16.115	132736850	1.11	58104845	1120-28-1	Eicosanoic acid, methyl ester
7	17.032	1914241	0.02	1051906	1731-88-0	Tridecanoic acid, methyl ester
8	17.494	3292573	0.03	2002001	1731-88-0	Tridecanoic acid, methyl ester
9	18.212	11501886	0.10	6130875	124-10-7	Methyl tetradecanoate
10	18.349	6112888	0.05	3237873	2777-58-4	6-Octadecenoic acid, methyl ester, (Z)-
11	18.486	42695049	0.36	25313537	56219-6-8	Methyl myristoleate
12	18.608	386002971	3.24	133167793	5129-66-8	Tetradecanoic acid, 12-methyl-, methyl ester
13	19.190	17418381	0.15	10854673	5129-60-2	Pentadecanoic acid, 14-methyl-, methyl ester
14	19.270	35805066	0.30	21749583	5129-58-8	Tridecanoic acid, 12-methyl-, methyl ester
15	19.309	11212009	0.09	8113026	2777-58-4	6-Octadecenoic acid, methyl ester, (Z)-
16	19.389	3560210	0.03	1872917	112-62-9	9-Octadecenoic acid (Z)-, methyl ester
17	19.516	73496410	0.62	44503891	7132-64-1	Pentadecanoic acid, methyl ester
18	20.047	18677519	0.16	10557932	5129-60-2	Pentadecanoic acid, 14-methyl-, methyl ester
19	20.125	2725887	0.02	1811978	16106-3-9	7,10-Hexadecadienoic acid, methyl ester
20	20.204	220555324	1.85	89037273	112-62-9	9-Octadecenoic acid (Z)-, methyl ester
21	20.428	2104752859	17.68	132121926	18951-36-5	Octadecanoic acid, 3-hydroxy-2-tetradecyl-, m
22	20.525	4044855	0.03	1558629	56134-3-3	1,8,11-Heptadecatriene, (Z,Z)-
23	20.818	20122845	0.17	10766022	6064-90-0	Heneicosanoic acid, methyl ester
24	20.889	24443058	0.21	13493621	929-77-1	Docosanoic acid, methyl ester
25	20.944	29695409	0.25	14732398	112-62-9	9-Octadecenoic acid (Z)-, methyl ester
26	21.085	56378512	0.47	27334703	1731-92-6	Heptadecanoic acid, methyl ester
27	21.408	7605494	0.06	1794204	18202-28-3	17-Octadecen-14-yn-1-ol
28	21.775	6776299637	56.91	685252431	142-77-8	Oleic acid, butyl ester
29	21.837	606453906	5.09	306644965	2490-23-5	Heptadecanoic acid, 14-methyl-, methyl ester
30	21.941	74637123	0.63	37404386	13038-47-6	9,11-Octadecadienoic acid, methyl ester, (E,E)
31	22.113	8746424	0.07	2939721	2566-97-4	9,12-Octadecadienoic acid, methyl ester, (E,E)
32	22.189	52381231	0.44	34583383	13038-47-6	9,11-Octadecadienoic acid, methyl ester, (E,E)
33	22.280	9107403	0.08	4473528	112-62-9	9-Octadecenoic acid (Z)-, methyl ester
34	22.414	7192473	0.06	4845561	5129-60-2	Pentadecanoic acid, 14-methyl-, methyl ester
35	22.600	3228350	0.03	781190	301-0-8	9,12,15-Octadecatrienoic acid, methyl ester, (Z
36	22.726	3233612	0.03	1683441	2566-89-4	5,8,11,14-Eicosatetraenoic acid, methyl ester,
37	22.823	21304175	0.18	5663784	56847-2-0	8,11,14-Docosatrienoic acid, methyl ester
38	22.947	242309312	2.04	116588983	112-62-9	9-Octadecenoic acid (Z)-, methyl ester
39	23.094	257282138	2.16	125852343	55124-96-4	Heptadecanoic acid, 15-ethyl-, methyl ester
40	23.368	4028729	0.03	1925512	18634-87-2	Pipataline
41	23.561	2788025	0.02	1262267	7459-33-8	9,12-Octadecadienoyl chloride, (Z,Z)-
42	23.864	5232563	0.04	2515179	6064-90-0	Heneicosanoic acid, methyl ester
43	24.384	16180831	0.14	5393323	7459-33-8	9,12-Octadecadienoyl chloride, (Z,Z)-
44	24.595	20371924	0.17	7112761	1120-34-9	13-Docosenoic acid, methyl ester, (Z)-
45	24.795	121300275	1.02	44403370	929-77-1	Docosanoic acid, methyl ester
46	25.914	14647316	0.12	4413589	929-77-1	Docosanoic acid, methyl ester
47	27.000	5516784	0.05	1582868	56554-33-7	15-Tetracosenoic acid, methyl ester
48	27.303	178789580	1.50	43492590	6064-90-0	Heneicosanoic acid, methyl ester
49	28.988	10392295	0.09	2289411	6064-90-0	Heneicosanoic acid, methyl ester
50	31.101	40879040	0.34	6499976	5802-82-4	Hexacosanoic acid, methyl ester
		11906399791	100.00	2161411540		

FIG 2 GCMS Analysis report of Bilva Taila

Table :7: Retention time area percentage and possible medicinal role of few compounds *Pharmacological activities listed in the table have been compiled from the following articles. Mishra et al. [7], Ansarali S et al. [8], Mercola et al. [9], Sharma S et al. [10], Starlin T et al. [11], Achi N et al. [12], Bishop CA et al. [13], Ogo O et al. [14]

slno	Retention time	Name of compound	Mol.formula	Mol weight	Library used	Area %	Pharmacological activities *
1	17.494	Tridecanoic acid methyl ester	C14H28O2	228	NIST 20	0.03	Antimicrobial Anti inflammatory
2	18.608	Tetradecanoic acid 12-methyl -methyl ester	C15H30O2	242	NIST 20	3.24	antimicrobial
3	19.190	Pentadecanoic acid 14-methyl-methyl ester	C17H34O2	270	NIST 20	0.15	Antimicrobial antioxidant Immune modu;lator
4	21.085	Heptadecanoic acid,15-ethyl-methy ester	C20H40O2	312	NIST 20	0.47	Antimicrobial antioxidant
5	22.947	9-Octadecanoic acid - methyl ester	C19H36O2	296	NIST 20	2.04	Antimicrobial
6	16.115	Eicosanoic acid,methyl ester	C21H42O2	326	NIST 20	1.11	Antimicrobial
7	27.303	Heneicosanoic acid	C22H44O2	340	NIST 20	1.50	Antimicrobial
8	24.795	Docasanoic acid methyl ester	C23H46O2	354	NIST 20	1.02	Antimicrobial
9	21.775	Oleic acid	C19H36O2	296	NIST 20	56.91	Antimicrobial Anti inflammatory Antioxidant Enhances drug penetration
10	22.114	Linoleic acid	C18H31ClO	298	NIST 20	0.07	Antimicrobial Antiinflammatory Antioxidant
11	22.600	Linolenic acid	C19H32O2	292	NIST 20	0.03	Antiinflammatory
12	22.726	5,8,11,14-eicosatetraenoicacid methyl ester	C21H34O2	318	NIST 20	0.03	Neuroprotective

DISCUSSION

Discussion on Physico-chemical parameters

Physico chemical parameter results obtained was compared with that of standard value for oil as per API. Loss on drying at 110 ^{o}C suggest the amount of water and volatile substance lost when the substance is heated under specified condition. While compairing with other oils the result 6.910% seems to be higher but it remains within the acceptable limits. It can be due to the presence of volatile phytoconstituents extracted during the preparation process. [15]

The refractive index of Bilva taila at 40^{0}C is 1.480. It comes under the acceptable range for medicated oils which means no adulteration. [16] The acid value is 4.127 which is as per the acceptable API limits.[17] It indicates the presence of small amount of free fatty acids and suggests minimal hydrolytic degradation and reflects good stability of the formulation. It has to be properly stored to prevent further oxidation and maintaining quality.

The iodine value is 75.94 indicates a moderate degree of unsaturation.[18] As per standards oils having iodine values below 100 are non-drying oil they have better oxidative stability which suggests suitability for prolonged storage and therapeutic use.

The saponification value of Bilva taila is 199.37 which indicates the presence of short to medium chain fatty acids.[19] A high saponification value means the oil is lighter and facilitate better absorption and spread ability over mucosal surface which helps for efficient delivery of active principles. The specific gravity of Bilva taila is 0.91. the lower density suggests lesser moisture content and indicates that the oil is free from adulteration. [20]

Qualitative test showed absence of microorganism and no any growth was noted in microbial limit test which suggests safety and stability of Taila as per API standards. Test for heavy metals showed less than 0.1ppm of lead, arsenic, cadmium and mercury which are under permissible limit by API. [21]

All the analytical reports of Bilva Taila suggests that the formulation is having good quality, stability and suitability for therapeutic application. From the observed value it can be concluded that the drug is non adulterated, relatively resistant to oxidative deterioration, have effective absorption of active constituents. Thus, supporting its use for therapeutic purpose.

Discussion on GCMS analysis of Bilva taila.

The GCMS analysis of Bilva Taila identified 50 compounds. All the identified compounds are given in figure1. The major identified compounds are Oleic acid, butyl ester with an area of 56.91%, followed by Octadecanoic acid,3-hydroxy-2-tetradecyl methyl ester 17.68%. Few compounds are identified more than once at different peak. Tridecanoic acid methyl ester is having two retention time at 17.032 and 17.494 with area percentage of 0.02 and 0.03. Pentadecanoic acid ,14-methyl-, methylester with three retention time 19.190, 20.047, 22.414 and area percentage 0.15, 0.16, 0.06. 9-Octadecanoic acid, methy ester with retention time 19.389, 20.204 and area percentage 0.03, 1.85. Heneicosanoic acid, methy ester with retention time 20.818, 27.303,28.988 and area percentage 0.17,1.50,0.09. Docosanoic acid, methyl ester at retention time 20.889,24.795,25.914 and area percentage 0.21,1.02,0.12. 9,11 octadecadienoic acid, methyl ester, (E, E) with retention time 21.941, 22.189 and area percentage 0.63 ,0.44.

Hexanoic acid methyl ester, Octanoic acid methyl ester, decanoic acid methyl ester, Tridecanoic acid and methyl tetradecanoate belong to medium chain fatty acid esters. Saturated long chain fatty acid esters include Eicosanoic acid methyl ester Pentadecanoic acid and its branched derivaties. Heptadecanoic acid and its derivatives. Various combinations of octadecenoic acid (z) esters, Oleic acid derivatives, linoleic and Linolenic acid esters belong to the group of unsaturated fatty acid esters. Branched and methylated fatty acid includes Tetradecanoic acid 12-methyl methyl esters, Pentadecanoic acid 14- methyl methyl ester, Tridecanoic acid 12 methyl methyl esters. Heneicosanoic acid, Docosanoic acid, Tetracosenoic acid and Hexacosanoic acid comes under very long chain fatty acids. Poly unsaturated fatty acids include Octadecatrienoic acid, Eicosatetraenoic acid, Docosatrienoic acid.[22]

Fatty acid methyl esters like Tridecanoic acid, Tetradecanoic acid, Pentadecanoic acid, Hexadecanoic acid, Heptadecanoic acid, Octadecanoic acid, Eicosanoic acid, Heneicosanoicacid, Docosanoic acid, Oleic acid, Linoleic acid, Linolenic acid have antimicrobial activity.[23] These compounds have a positive effect on certain gram positive and gram-negative bacteria's and candida. [24]

Compounds like Oleic acid (9-octadecenoic acid), Linoleic acid (9,12-Octadecadienoic acid) is having anti-inflammatory properties. Oleic acid can decrease the production of IL-1β, IL-6 and CINC-2 αβ by resident macrophages and Linoleic acid decrease the production of IL-1β, IL-6 and VEGF. [25] Few studies also reveal the effect of PUFA in maintaining hearing loss by anti-inflammatory actions. [26]

Pentadecanoic acid, Heptadecanoic acid,[27] Oleic acid, Linoleic acid, Eicosanoic acid are having antioxidant property. [28] Fatty acids rich in anti-oxidants has increased hearing threshold [29] and also helped in preventing age related hearing loss. [30] Pentadecanoic acid can integrate into phospholipid membrane at cellular level which helps in withstanding lipid peroxidation by stabilising membranes against oxidative damage and may slow down premature cellular senescence. [31]

5,8,11,14- Eicosatetraenoic acid, methyl ester is having neuroprotective effect. [32] Oleic acid can deal with amyloidosis and can decrease 7-ketocholesterol which can be seen in neurodegenerative cases. [33]

Discussion on Drugs in Bilva taila.

Apakva Bilva Phala Majja

Apakva Bilva Phala Majja is having Katu, Tikta, Kashaya Rasa. Laghu, Ruksha, Tikshna Guna, Ushna Virya, Katu Vipaka and Vata Kapha Shamaka Karma.[34] Vata kapha Shamaka property of the fruit helps in the management of Badhirya. It is a potent drug processing anti- inflammatory, anti-microbial, mast cell stabilization and detoxifying action. The pulp of the Bael fruit is rich in bioactive substances such as carotenoids, phenolics, alkaloids, pectins, tannins, coumarins, flavonoids, and terpenoids. Tannin has antioxidant and anti -inflammatory action. Terpenoids are anti- bacterial and anti-fungal. Flavinoids are antinociceptive. [35]

Tila Taila

It is Madhura kashya in Rasa, Guru, Snighdha, Sukshma, Vyavayi Guna.Ushna Virya, Madhura Vipaka and Vata Shamaka Karma.[34] kim etal in his studies directly suggest that Sesamin found in Sesame oil is found to be effective in auditory impairment otic cells increased in numbers after using sesame oil.sesame oil was also found to be effective in noise induced hearing loss in a study accessed by auditory brain stem respone where a hearing thresh hold shift was found.In a study related to Tecta hearing related gene the expression of Tecta increases in the apex area of cochlear hair cells as they grow after using sesame oil.[36]

Aja ksheera

Aja ksheera is Kashaya Madhura Rasa, Laghu, Snigdha Guna, Sheeta Virya, Madhura Vipaka , Tridosha Shamaka.[34] Various researches shows that goat milk proteins like casein,whey protein minor proteins like lysozyme lactoperoxidase and serum albumin are having anti-inflammatory and antioxidant effect.[37]

Go mutra

It is Katu Tikta Lavana Rasa, Thikshna Laghu Ruksha Guna, Ushna Virya, Katu Vipaka, Kapha Vata Shamaka.[34] Cow's urine is having antimicrobial and germicidal properties due to the presence of carbolic acid, phenols, calcium, manganese. It has the ability to prevent the development of antibacterial resistance by blocking the R factor. Phenolic acid has anti-fungal effects. Uric acid present in cow's urine have antioxidant property which may prevent the process of aging and repairs the damaged DNA.[38]

Disscussion on probable mode of action of Bilva Taila

Antimicrobial action

Otitis externa, media and labyrinthitis are caused by microbes. Staphylococcus, Streptococcus, Pseudomonas, Aspergillus and candida species are common among them.[39] Otitis externa and media if not treated properly can lead to development of conductive hearing loss by causing inflammation, fluid accumulation, perforation to tympanic membrane or by mechanical disruption to ossicles. [40] These infections can cross middle ear and reach inner ear leading to sensorineural hearing loss by damaging cochlear and vestibular structures. [41] Medium chain fatty acids present in Bilva Taila has antibacterial properties. [42]

Gomutra is having Krimighna property studies reveals that its antimicrobial activity is compairable with standard drugs, it is also found to be best against resistant strains. [43] Bilva phala extracts is found to be effective against streptococcus, staphylococcus, aspergillus, candida and to be effective in the early stages of viral replication thus having anti-bacterial anti-fungal and anti-viral properties. It is found to be comparable with drug Ciprofloxacin. [44] Tila taila is also having anti-microbial properties [45] and is effective against Staphylococcus, Streptococcus and Fungi. [46]. Krimighna properties of the drug suggest its effectiveness in the infectious stage and arresting of the progress thus preventing Badhirya.

Anti-inflammatory action

Otitis externa will lead to development of various inflammatory responses by causing damage to the epithelium and increasing the pH level by accumulating moisture. [47] In otitis media the inflammatory process will build up mucosal secretion in middle ear. [48] Both if untreated will end up in conductive hearing loss. Inflammation has a key role in manifestation of SNHL [49} exposure to large sounds also enhances the inflammatory changes and ends up in SNHL.[50]

The invivo and invitro analysis of Tila Taila has proven that it has anti-inflammatory action. Some of the chemical constituents present in the oil can stop the propagation of inflammatory cytokines and mediators which help in the action.[51] Aja ksheera is anti-inflammatory it helps in inhibition of TNF- α and IL-1βand IL-10 secretion which plays a key role in developing inflammation.[52] The unripe fruits of Bilva are anti-inflammtory and also have mast cell stabilizing action. [53] The contents in Gomutra helps to suppress inflammatory enzymes and their products thus subsiding inflammation. [54]

The build-up secretions can be considered as accumulation of Kapha and the Badhirya developed is Vata kaphaja type since the accumulated Kapha obstructs the normal pathway of Vata. [55] The ingredients in Bilva taila such

as Gomutra and Tila Taila are Kaphagna and because of its contents Bilva taila itself is Ushna, Laghu and Kapha hara so it can do Kaphavilayana and expels the Kapha thus overcoming the Avarana.

Antioxidant property

Oxidative stress can lead to SNHL.[56] Tila Taila is also rich in antioxidants.[57] Studies suggest that the presence of antioxidants in Bilva Phala helps to treat cellular damage caused by free radical. [58] The antioxidant property of cow's urine is attributed to the free radical scavenging activity of the urine components which slow down the process of ageing even after minimal processing. [59]

Neuroprotective effect

An invivo study on Tila Taila suggests that the components in Sesame oil have the ability to protect auditory hair cells and reversal of its impairment it also helped in decreasing hearing threshold.[60] Bilva Phala also has ability to alter cellular mechanism that causes neuronal damage. [61] Presents of lot of micronutrients and omega 3 fatty acid makes goat milk a best neuroprotector.[62]

Anti degenerative property

Long standing inflammation will lead to sclerotic and fibrotic changes in tympanic membrane, otosclerosis and fixation in ossicular chain which leads to mechanical disruption in conducting sound waves due to loss of elasticity and loss of movements being anti-inflammatory and anti-oxidant it can prevent or decrease the degenerative effect. The Snehana effect of Taila helps in Dathukshya condition and improves the situation.

Drug penetration

Studies suggest that sesamum oil enhances drug penetration and permeation. [63] Various studies on Bilva Phala suggests that it can be used as adjuvant with other drugs to increase effectiveness. [64] The Sukshma and Vyavayi Guna of Tila Taila also enhances the drug penetration.Oleic acid can enhance the penetration of the medicine and can facilitate the transport of the drugs to the inner ear by enhancing the permeability of round window. [65]

CONCLUSION

By doing Pharmacognostic and physiochemical evaluation of Bilva Taila and analysing the results it is found that the drug is analytically stable and safe for use.The formulation was having the API standards.After analysing the GC-MS report the constituents of the drug is mainly fatty acid components. Based on the previous studies the compounds pocess anti-microbial, anti-inflammatory, anti-oxidant, anti-degenerative, neuroprotective effect which validates the indication of Bilvataila in Vatja and VataKaphaja types of Badhirya and also as a protective or preventive medicine.

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