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INSIGHTS INTO PHYTOCHEMISTRY AND THERAPEUTIC APPLICATIONS OF AN AYURVEDA FORMULATION IN HYPOTHYROIDISM

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ABSTRACT

Ayurveda has gained a lot of attention by its effectiveness and popularity. This medical system originated centuries ago with medicines prepared as per needs of the individual cases. In the present era, where medicines are manufactured on a larger scale, it requires standardisation in order to keep up the quality and efficacy. This study is aimed to standardize a compound Ayurveda formulation intended for managing the clinical symptoms of hypothyroidism by its organoleptic evaluation, physicochemical and chromatographic analysis. The physico-chemical analysis of the tablets was performed as per API standards. The formulation was subjected to TLC-HPTLC fingerprinting. All physicochemical parameters like uniformity of weight, disintegration time, friability, hardness were done as per API protocol and hence the results obtained can be used for standardisation of this particular Gulika. TLC of compound formulation was developed in Toluene : chloroform : methanol (8:3:1) solvent system resulted in maximum compound separation and observed for significant spots. HPTLC analysis showed presence of various compounds whose structural identification could not be confirmed with matching R_f of standard compounds.

Key words: Hypothyroidism, Ayurveda, TLC-HPTLC fingerprinting, Standardisation

INTRODUCTION:

Ayurveda medicines are noted for their multidimensional actions and therapeutic applications. Every herb used as a medicine in Ayurveda is expected to have some variations in the quality and quantity of active principles. This might be attributed to a wide range of variations possible due to geographycal, seasonal and during processing. Thus is becomes an essential practice to analyse and standardise every Ayurvedic medicine which may either currently in practice or a new combination which gains popularity. In order to maintain the quality and efficacy of an Ayurvedic medicine throughout the world its standardisation is a must. The present study envisages to standardize a compound Ayurveda formulation prescribed for managing the clinical symptoms of hypothyroidism by its organoleptic evaluation, physicochemical and chromatographic (Thin layer chromatography- TLC and High-Performance Thin Layer Chromatography-HPTLC) analysis. Each of the ingredients of the composition were studied for the phytochemical composition and probable efficacy in hypothyroidism by a thorough review of the published data from peer reviewed journals. The ingredients of the formulation and their probable utility in managing the clinical symptoms od hypothyroidism have been well described below:

Aswagandha: Root extract of *Withania somnifera* of Solanaceae family recognised as Aswagandha has been proven to be effective in stimulating thyroid function in female mice especially by increasing serum T4 levels **[1]**. *Withania somnifera* root extracts are rich in steroidal lactones like withanolides and withaferins, alkaloids and saponins **[2]**. The adaptogenic, antiinflammatory and anxiolytic effects of the root extracts are well explored and their role on endocrine pathways and Central nervous system can't be overlooked.

Guggulu: The oleogum resin that is tapped from the bark of Commiphoramukul of Bursaraceae family is used as Guggulu in Ayurveda. Phytochemical analysis shows that this oleogum resin is rich in volatile oil composed of terpinoidal components, flavonoids, steroids, lignans, guggulutetrols, amino acids and sugars **[3]**. Administration of Guggulu in female mice with induced hypothyroidism revealed that the herb was efficient in increasing serum T3 level alongside decrease of Lipid Peroxidation in Liver **[4]**. Guggulu is also effective in

hypercholesteremia and has anti-inflammatory actions by virtue of guggulusterone which helps it augment thyroid function. The exudate has also been seen to augment the Basal Metabolic Rate in hypothyroid individuals **[9]**. Kanchanaraguggulu, mahayogarajaguggulu, Chandraprabhavati are major formulations having Commiphoramukul which are used in managing hypothyroidism.

Brahmi: the whole plant of Bacopamonnieri (L.) Wettst. of Scrophulariaceae family is accepted as Brahmi in Ayurveda. Studies show that the herb stimulates thyroid activity by increasing the amount of T4 levels **[5]**. Phenols, cardiac glycoside, tannin, alkaloid and saponin are found in abundance in Brahmi **[6, 7]**.

Punarnava: Root of *Boerrhavia diffusa* of Scrophulariaceae family recognised as Punarnava in Ayurveda is composed mainly of steroids, flavonoids, xanthones, purine nucleoside and lignans **[8]**. Popularly Ayurveda physicians prescribe Punarnava in cases of thyroid swelling taking in view its exceptional diuretic and anti-inflammatory properties **[9]**.

Makandi: consists of the whole plant or root of Coleus forskohlii (Willd.) Briq. of Lamiaceae family. It is a source of essential oils, diterpenes like "Forskolin", diterpenoids flavanoids etc. which attribute to its antihypertensive, cardioprotective, antioxidant, Anti asthmatic and various other pharmacological actions **[10]**.

Pippali: The fruit of Piper longum of piperaceae called Pippali in Ayurveda exhibits multitude of pharmacological properties like immunomodulatory, , anti-hyperlipidemic, anti-platelet, anti-inflammatory, antihypertensive, antioxidant, Anti asthmatic etc owing to the presence of phytochmecals like alkaloids (piperine and piperlongumine), flavonoids, steroids and essential oils **[11]**.

Guduchi: Guduchi (*Tinospora cordifolia*) belonging to family Menispermaceae is the best rejuvenative (rasayana) in Ayurveda. It is an ample source of phytochemicals like alkalois, glycosides, diterpenoid lactones, steroids etc. that bestow anti-inflammatory, anti-oxidant, adaptogen, hypolipidemic and immunomodulatory actions **[12]**. These properties of Guduchi make it worthy in managing hypothyroidism and augmenting the metabolism in the individual.

Nimba: The leaf, stembark, fruit and root of *Azadiracta indica* of Meliaceae family are used in Ayurveda as Nimba. Azadirachtin and nimbolide in Neem plays role as free radical scavenging properties due to rich source of antioxidants **[13]**. Nimba has

profound anti-inflammatory property via regulation of proinflammatory enzyme activities including cyclooxygenase (COX), and lipoxygenase (LOX) enzyme **[13]**. It has strong immunomodulatory action.

MATERIALS AND METHODS:

Study drug: The constituent herbs of the formulation were collected from Vaidyaratnam Oushadhasala Thikattussery, Ollur, Thrissur district Kerala, and the tablets were manufactured and authenticated by the in house quality control (QC). The physico-chemical analysis of the tablets was performed as per API standards. The formulation was subjected to TLC-HPTLC fingerprinting at Carekeralam pvt. Ltd, Koratty Thrissur, Kerala.

Methodology:

AVERAGE WEIGHT:

Randomly selected 20 tablets were weighed and average weight was calculated.

Disintegrating time [11]: This test determines whether the tablet disintegrate within a prescribed time when placed in a liquid medium under the prescribed experimental conditions. The tank of the disintegration apparatus was filled with distilled water up to the mark. 750 ml of distilled water in each of the 1000 ml beaker was taken. The timer of the instrument was set for 60 minutes. The temperature of water in beakers to 37^0C and that of water in the main tank to 37^0C was maintained. One tablet was introduced into each tube and added a disk to each tube. The assembly was suspended in the beaker containing water and the apparatus was operated. The time duration at which the tablet disintegrates was noted.

Friability [11]: One of the commonly employed test to measure the ability of tablets to withstand mechanical stresses determines their resistance to chipping and surface abrasion by tumbling them in a rotating drum. The percentage weight loss after tumbling is referred to as the friability of the tablets. The friability test is conducted in the Roche friability apparatus by taking 20 tablets. This consists of a plastic drum that revolves at 25rpm, dropping the tablets through six inches in the friabilator to undergo shock, which is then operated for 100 revolutions. The tablets are reweighed. The tablet that lose less than1.0% of the tablet weight are considered as acceptable.

Hardness [11]: Hardness is the measure of the mechanical integrity of the tablets. It is the force required to break the tablets in a specific plan. The randomly selected tablets were tested individually using Pfizer tablet hardness tester. The tablet was held vertically in between the jaws of hardness tester. The

force applied on the edge of the pill was gradually increased by pressing the jaws with the help of hand until the tablet breaks. The reading was recorded, and the average hardness of each group was calculated separately.

INSTRUMENTS AND CHROMATOGRAPHIC TECHNIQUE:

Thin Layer Chromatography (TLC) was done and Rf values were calculated. Further HPTLC analysis was carried out by the CAMAG HPTLC system (Switzerland). Samples were applied using CAMAG ATS 4 auto sampler on aluminum backed pre-coated silica gel $60F_{254}$ HPTLC plate (Merck India). Mobile phase was optimized as toluene, ethyl acetate, and methanol in the ratio of 7:3:1. The chromatogram was developed in a saturated Twin Trough chromatographic chamber (Camag, Switzerland) and was visualized under UV-chamber (254 and 366 nm) and in visible light after derivatizing with anisaldehyde sulfuric acid reagent followed by heating at 105 °C for 5 min.

RESULTS:

Results of Organoleptic analysis are given in **Table 1**.

The tablet was subjected to Physiocochemical Analysis as per API standards and the results are given below in **Table 2**.

RESULTS OF TLC and HPTLC

Rapid chromatographic method has been developed for the chemical fingerprinting of selected medicines by modern high-performance thin-layer chromatography. The optimized mobile phase provided good resolution under various documentation systems such as UV-254, 366, and visible light. Chromatogram and 3D-illustrated display are presented in **Figure 1**.

HPTLC analysis showed presence of various compounds whose structural identification could not be confirmed with matching R_f of standard compounds. The data obtained is presented in the in **Figures 2, 3**.

Table 1: Results of Organoleptic analysis:	
Parameters	**results**
Taste	**Bitter**
Shape	**Biconvex**
Size	**Thickness- 4.83mm, Diameter- 12.mm**
Odour	**Faint**
Colour	**Black**

Table 2: Results of Physicochemical Analysis		
PARAMETERS TESTED	**RESULTS**	**REFERENCE METHOD**
Disintegration	**32 minutes**	**API**
Friability	**Complies**	**API**
Hardness	**6 kg**	**API**
Average weight	**125 mg**	**API**
Uniformity of weight	**Complies**	**API**

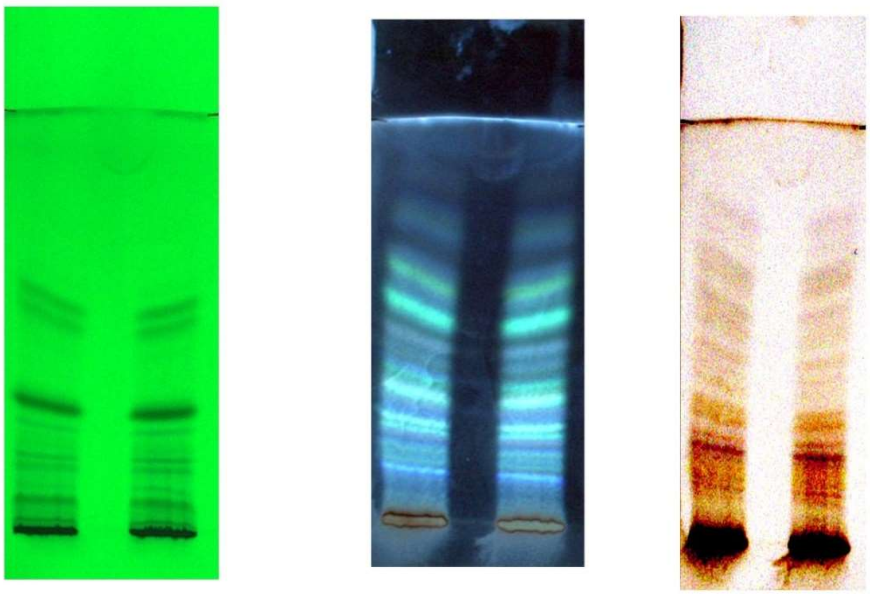
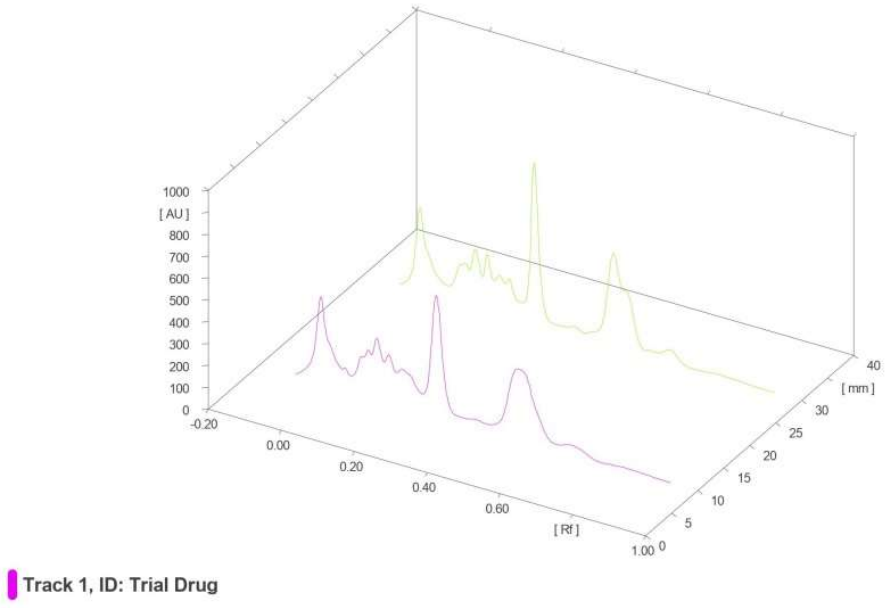


Figure 1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Track 1, ID: Trial Drug
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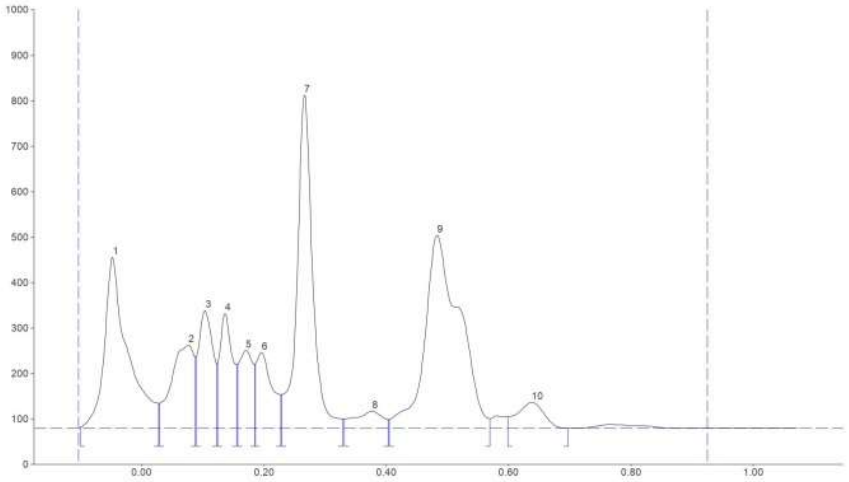
0.20
0.40
0.60
0.80
1.00

Figure 2

winCATS Planar Chromatography Manager

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.10	1.4	-0.03	388.2	17.35	0.02	84.7	12115.7	17.34	unknown *
2	0.03	84.8	0.03	93.0	4.16	0.05	65.2	1304.3	1.87	unknown *
3	0.05	65.4	0.10	205.8	9.20	0.10	188.7	5608.5	8.03	unknown *
4	0.11	189.4	0.12	272.0	12.16	0.14	174.1	5327.9	7.62	unknown *
5	0.14	175.9	0.15	212.1	9.48	0.17	141.2	3965.3	5.67	unknown *
6	0.17	141.4	0.19	163.0	7.29	0.24	77.3	6396.7	9.15	unknown *
7	0.25	77.6	0.28	544.8	24.35	0.35	21.6	14019.7	20.06	unknown *
8	0.42	24.6	0.51	317.2	14.18	0.61	24.3	19132.3	27.38	unknown *
9	0.61	24.3	0.65	41.1	1.84	0.73	0.0	2016.9	2.89	unknown *

Track 2, ID: Trial Drug



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.10	2.9	-0.05	376.3	14.13	0.03	55.6	11424.8	16.45	unknown *
2	0.03	55.8	0.08	183.2	6.88	0.09	157.3	5398.6	7.77	unknown *
3	0.09	158.9	0.10	258.9	9.72	0.12	141.1	4897.1	7.05	unknown *
4	0.13	144.1	0.14	253.1	9.50	0.16	141.4	4172.7	6.01	unknown *
5	0.16	142.2	0.17	171.2	6.43	0.19	141.3	3122.8	4.50	unknown *
6	0.19	142.6	0.20	167.3	6.28	0.23	74.7	3439.9	4.95	unknown *
7	0.23	74.7	0.27	733.2	27.52	0.33	21.0	14668.1	21.11	unknown *
8	0.33	21.2	0.38	38.1	1.43	0.40	19.5	1380.2	1.99	unknown *
9	0.41	19.7	0.48	425.1	15.96	0.57	21.4	18998.9	27.35	unknown *
10	0.60	26.0	0.64	57.4	2.15	0.70	0.1	1968.5	2.83	unknown *

Visualizer Document - Plate state Developed

Figure 3

DISCUSSION

All physicochemical parameters like uniformity of weight, disintegration time, friability, hardness were done as per API protocol and hence the results obtained can be used for standardisation of this particular Gulika. TLC of compound formulation was developed in the solvent system Toluene: chloroform: methanol (8:3:1). This particular solvent system resulted in

maximum compound separation and hence finished product was also developed in the same solvent system and observed for significant spots. The same solvent system was taken for HPTLC. Chromatography for individual herbs was not done leading to a limitation in comparing the composition of individual drugs and the formulation. Standardization of the Ayurveda compound is need of the hour and the data obtained in the present study may be used as reference for quality standards for this formulation used in managing clinical symptoms of hypothyroidism.

CONCLUSION

The unique formulation is constituents of herbs that have been proven to be efficient in tackling the clinical symptoms of hypothyroidism and thereby promise to improve the quality of life of the individuals. A trial for analysing the efficacy of this formulation in clinical setting will follow this initial study for standardisation of the compound. The results of analysis of the physico-chemical characteristics and TLC, HPTLC fingerprint profiles can be made useful for deciding the identity, purity and strength of the polyherbal formulation.

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