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FORMULATION AND EVALUATION OF POLYHERBAL TABLET FOR THE TREATMENT OF THYROID DISORDER

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ABSTRACT

Objective: The objective of the present study was to formulate polyherbal tablet for the treatment of thyroid disorders and to carry out evaluation of prepared polyherbal tablet. **Methodology:** Kanchanara, Guggul, Jatamansi, Trikatu, Brahmi, Amla, Amulthus, Chandrashoor, Methi and Giloy extracts were selected for the formulation of hypothyroid tablets to overcome the symptoms associated with thyroid disorders. The ethanolic extracts were subjected to qualitative chemical investigation to test for the presence of various phytochemical constituents in the extract and formulate hypothyroid tablet by wet granulation technique. Then film coating of tablets were carried out by Aquadry brown for taste masking and physical protection of tablets. Granules, uncoated tablet and coated tablets were evaluated for tests i.e. flow property, friability, dimension, hardness, disintegration time and drug content by HPTLC method. Microbial testing and stability study were performed to check the quality of product. Tablets were evaluated for determining its effects in the regulation of hypothyroidism using Albino wistar rat model. Serum triiodothyronine, thyroxine and thyroid stimulating hormone concentrations were tested as the end parameters of thyroid function in the study. **Results:** The tablets were evaluated for various parameters and the product were found to be within standard ranges and stable at stability and microbial testing and improves hypothyroidism activity in Albino rats. **Conclusion:** The hypothyroid tablet can find its application as thyroid supplement which can be given easily and safely to patients suffering from thyroid disorders.

KEYWORDS: Thyroid disorder, hypothyroidism, chemical investigation, triiodothyronine, thyroxine, albino wistar.

INTRODUCTION

Worldwide about 25% of prescribed drugs are derived from plants.[1] The maximum use of herbal medicines is for chronic therapies and health promotion and prohibit to life-threatening conditions.[2] On the basis of data of community studies the prevalence of hyperthyroidism in in male is 0.2% and in female is 2%, and about 15% of hyperthyroidism patients occurring in patients above 60 year of age. Similarly hypothyroidism occurrence is of around 0.3% to 0.4%, which increases with age and most commonly found that females are affected.[3] Thyroid gland secretions are the 2 thyroid hormones Triiodothyronine or T3 and Thyroxin or T4 as well as calcitonin. The production of triiodothyronine (T3) and thyroxine (T4) in the thyroid gland is regulated through hypothalamus and pituitary gland by a complex feedback mechanism. For ensuring stable levels of thyroid hormones hypothalamus monitors circulating levels of thyroid hormones and it responds to low levels through releasing thyrotropin-releasing hormone (TRH). Thyroid stimulating hormone (TRH) is then stimulated by pituitary gland. When there is increase in level of thyroid hormone, TSH production decreases, which slows the release of new hormones from the thyroid gland.[4]

Hypothyroidism: Failure of thyroid gland, TRH, TSH deficiency, or both and scanty supply of iodine in diet lead to hypothyroidism.

Primary hypothyroidism: Lowered level of thyroid hormone along with raised TSH level in blood chances the primary hyperthyroidism; which usually stipulates the defective thyroid synthesis.

Secondary hypothyroidism: Low level of both TSH and thyroid hormone leads to secondary hyperthyroidism. This reflects that the pituitary gland is accountable for lowered thyroid functioning.

Hashimoto's disease: An autoimmune condition where antibodies bind to thyroid and avert the production of adequate levels of thyroid hormone. Additionally to antibodies binding with thyroid tissue, they may well

bind to the acid producing cells of the stomach and other glands like the adrenal glands, the pancreas gland.

Cretinism: Hypothyroid function since birth leads to cretinism. Lowered level of thyroid hormone may lead to mental retardation, dwarfism, and thyroid deficiency symptoms. Conventional Western medical treatment employs exogenous thyroid hormone.[5]

Hyperthyroidism: An elevated function of thyroid means hypothyroidism. Hyperthyroidism occurs when there is a hyper metabolic condition due to excessive level of thyroid hormone synthesis and secretion. Most commonly hyperthyroidism is called as "Graves's disease".[6-7]

MATERIAL AND METHOD

Procurement of plant extracts

Kanchanara (*Bauhinia variegate*), Guggul (*Commiphora mukul*), Jatamansi (*Nardostachys Jatamansi*), Trikatu (*Zingiber officinalis, Piper nigrum* and *Piper longum*), Brahmi (*Bacopa monnieri*), Amla (*Emblica officinalis*), Amulthus (*Cassia fistula*), Chandrashoor (*Lepidium sativum*), Methi (*Trigonella foenum-graecum*) and Giloy (*Tinospora cordifolia*) extract were procured from Phyto Life Sciences Pvt. Ltd. Ahemadabad, Gujrat.

Ingredients of tablet and their Pharmacological properties

Each batch of 1000 tablets was prepared which contains

1) **Kanchanara (*Bauhinia variegate*):** *Kanchnara* is considered as a drug of choice for *Granthivikara* and *Galaganda.* It has a balancing activity on the thyroxin production, increasing any deficient production and decreasing any excess. It also clears swellings in the neck and goiter. It is a specific herb for swollen lymph nodes, cervical adenitis, scrophularia or swollen glands in general.[8-9]
2) **Guggul (*Commiphora mukul*):** A compound of *commiphora*, oleoresin, and increases iodine re absorption by the thyroid gland and also increases the activity of thyroxidase. The *guggul* extract contains oleoresin. It has a strong thyroid-stimulating activity. It also increases the production of T3 by increasing the conversion of T4 to T3. It increases lipid peroxidation and also increases serum T3 level. Recent studies have shown that certain compounds of *guggul* resin have anti-inflammatory properties, lower cholesterol, and other blood lipids and support thyroid function in different ways.[10]
3) **Jatamansi (*Nardostachys Jatamansi*):** It was studied for the growth of hairs due to cancer treatment. The results confirmed hair growth promotion activities of this plant. The hair growth study was design not only to see effect of extract on hair growth but also on isolated fraction named as nardal, jatamansic acid and nardin.[11]
4) **Trikatu (*Zingiber officinalis, Piper nigrum* and *Piper longum*):** Trikatu powder contains three spices in equal proportions. Black pepper - *Piper nigrum*, Long pepper fruit - *Piper longum*, Ginger - *Zingiber officinalis*. It increases the absorption of ayurvedic medicines. It has hot potency, which means its intake results in production of heat in the body and increase digestive juices and bile salt secretion. Trikatu Churna has primary effect on stomach and increases digestive juices, which helps stimulating digestion. It also has influence on liver and increases production of bile salts. It stimulates secretion of bile from the gall bladder. It stimulates pancreas functions. Therefore, it affects overall digestive system.[12]
5) **Brahmi (*Bacopa monnieri*):** Stimulates thyroid activity by increasing the amount of T4 useful in treatment hypothyroidism.[13-14] The triterpenoid saponins and their bacosides are responsible for Bacopa's ability to enhance nerve impulse transmission. The bacosides aid in repair of damaged neurons by enhancing kinase activity, neuronal synthesis, and restoration of synaptic activity, and ultimately nerve impulse transmission.[15]
6) **Amla (*Emblica officinalis*):** Amla is one of the richest sources of vitamin-C and low molecular weight hydrolysable tannins which makes Amla a good antioxidant. The tannins of amla like emblicanin-A (37%), emblicanin-B (33%), punigluconin and pedunculagin are reported to provide protection against oxygen radical included haemolysis of rat peripheral blood erythrocytes. [16] The mechanism behind antioxidant activity is due to the recycling of sugar moiety and conversion of the polyphenol into medium and high molecular weight tannins. The powerful antioxidant ellagic acid, present in amla, can inhibit mutations in genes and repairs the chromosomal abnormalities. Amla inhibits the growth and spread of various cancers like breast, uterus, pancreas, stomach and liver cancers.[17]
7) **Amulthus (*Cassia fistula*):** The purgative action of fruit pulp of *Cassia fistula* is due to presence of anthraquinone, known to have purgative action.[18]
8) **Chandrashoor (*Lepidium sativum*):** It is the rich source of non-haeme iron (iron found in haemoglobin) which is an easily absorbed dietary iron. It helps to increase the hemoglobin level in blood. When taken regularly, it helps to alleviate anemia. It is advisable to have vitamin C half an hour after consumption of these seeds as it enhances iron absorption. L-ascorbic acid facilitates iron absorption by forming a chelate with ferric iron at acid pH converting them to ferrous state that remains soluble at the alkaline pH of the duodenum which gets easily absorbed.[19-20]
9) **Methi (*Trigonella foenum graecum*):** The steroidal saponins (diosgenin, yamogenin, tigogenin and neotigogenin) are thought to inhibit cholesterol absorption and synthesis and hence its potential role in arteriosclerosis. Clinical studies demonstrated a statistically significant decline in human serum total

cholesterol, triglycerides and LDL cholesterol by fenugreek consumption.[21-31]

10) **Giloy (*Tinospora cordifolia*):** This is due to the alkaloidal constituents (choline, palmatine, tetrahydropalmatine and magnoflorine), epicatechin, and an aromatic glycoside, secoisolariciresinol. An arabinogalactan polysaccharide from *Tinospora cordifolia* showed protection against iron-mediated lipid peroxidation of rat brain homogenate using lipid hydroperoxide (LOOH), possibly due to its high reactivity towards DPPH, superoxide radicals and hydroxyl radicals. Our study showed that the alkaloidal fraction (mainly palmatine, jatrorrhizine and magnoflorine) had better antioxidant activity in the DPPH inhibition assay than columbin, in a dose dependent manner.[32-35]

Phytochemical investigation: The ethanolic extracts were subjected to qualitative phytochemical screening to check the presence of various phytoconstituents and standardized all the extracts. The various procedures were adopted to test the presence of various phytochemical constituents in the extract. Most important of these bioactive constituents of plants were steroids, terpenoids, flavanoids, alkaloids, tannins, saponins and glycosides.

Formulation procedure: All the above extracts were selected for the formulation of hypothyroid tablet to overcome the symptoms associated with thyroid disorders. The tablets were prepared by wet granulation technique. All the ingredients of table No. 1 were mixed homogenously. Excipients were added to the mixture for proper binding of the tablets. Then film coating of tablets were carried out by Aquadry brown for taste masking and physical protection of tablets. Aquadry brown caplet hypothyroidism tablet 855 mg was prepared in automatic tablet making machine. Weight after each pharmaceutical process was noted to observe the processing loss. About total 1000 to 1100 tablets were obtained from each sample batch 1, 2 and 3. Tablets were packed in air tight bottle and stored in cool dry place. All hygienic conditions were maintained during preparation of tablet. This procedure was implemented for preparation of three different batches of tablets.

Table 1: Formulation of Tablet

Sr. No.	Ingredients	Quantity for each tablet (mg)
1	Kanchanara extract	100
2	Guggul extract	80
3	Jatamansi extract	90
4	Trikatu extract	10
5	Brahmi extract	50
6	Amla extract	60
7	Amulthus extract	30
8	Chandrashoor extract	60
9	Methi extract	30
10	Giloy extract	50
11	Microcrystalline cellulose I.P. type 101	111
12	Dibasic calcium phosphate	80.4
13	Sodium benzoate I.P.	0.8
14	Potassium sorbate I.P.	0.8
15	Sodium starch glycolate I.P.	40
16	Crosspovidone XL10	22
17	Talc	10
18	Aerosil	5
19	Aquadry brown	0.05
20	Solvents: a) Isopropyl alcohol b) Methylene chloride	 0.3325 ml 0.6175 ml

In process checks: Various in process check parameters of granules were tested before tablet compression such as loss on drying, tapped and bulk density, % Carr's index, angle of repose and hausner ratio.

Evaluation of tablet: After tablet compression uncoated tablet were evaluated for parameters friability, hardness, tablet dimensions, weight variation, disintegration time, identification and coated tablets for hardness, tablet dimensions, weight variation, disintegration time and drug content by HPTLC.

Qualitative and Quantitative Estimation

HPTLC Instrumentation: A Camag HPTLC system was equipped with a Linomat V sample applicator, TLC Scanner 3, twin trough plate development chamber, 100 mL Hamilton Syringe and win CATS software.

Sample preparation

Reference Sample Preparation: Reference sample of quercetin and guggulsterones E and Z for *Bauhinia variegata* and *Commiphora mukul* content estimation was taken in 250 ml iodine flask with 30 ml methanol and refluxed for 45 min, cooled and filtered. The remaining residue was refluxed again twice with 15 ml of methanol for 25 min. Again cooled, filtered and combined the washing. Evaporated on water bath and dissolved the residue with 10 ml methanol in volumetric flask.

Test Sample Preparation: Weighed and powdered 20 tablets. Weighed accurately about 600.0 mg of powdered tablets in 250 ml of iodine flask and added 30 ml of methanol and refluxed for 30 mins. Then allowed it to cool & filter; further refluxed the content of the flask with 2x20 quantity of methanol; each time filtered and combined methanol washing and on water bath allowed to dry and residue is dissolved in volumetric flask in 10 ml methanol.

Mobile phase composition for Kanchanar (*Bauhinia variegate*) content estimation

Toluene: Ethyl acetate (7:3).

Mobile phase composition for Guggul (*Commiphora mukul*) content estimation

Hexane: Ethyl acetate (6:4)

HPTLC of tablet: All three samples batches of this formulation were studied for HPTLC and spots were observed. For Study 10µl layer was applied on HPTLC plate and at a distance of 10 cm, plate was developed by using mobile phase. The plate allowed to air dry after development and examined under UV light.

Microbial testing: This test has been performed to check microbial quality of tablets.

Stability study: The prepared tablets were packed in container and seal using aluminium foil and subjected to stability studies at 75% RH and 40 °C in stability chamber for a period of 3 months. After 3 months samples were withdrawn and evaluated for physical appearance, hardness, weight variation, disintegration time and drug content. It was found that the prepared tablet was stable at 40°C/75% RH, after 3 months.

Animal study

Experimental protocol design: Male and female albino wistar rats approximately 150-200 g in weight were used for screening which obtained from the animal house of the Kamla Nehru College of Pharmacy, Butibori, Nagpur. During the study period animals were fed with standard laboratory food. They had housed under standard condition of humidity-controlled environment (50±5 %) and temperature- (22±1°C) and had free access to food and water. The animal house has been approved by the committee for purpose of control and supervision of experimental animals (CPCSEA). (Registration number 1389/a/10/CPCSEA).

Animal protocol: Eighteen albino wistar rats (either sex) will be divided into three groups. Group- I will have 6 animals and group-II and III each will have 6 animals. While group 1, receiving 0.1 ml of normal saline served as control, animals of group 2 and 3 were treated for first 12 days with 10 mg/kg/day of Methimazole. From 13th day onwards, group 2 receiving 100 mg/kg/day of self made tablet formulation of *Bauhinia variegata* and group 3 receiving 0.5 mg/kg/day of Thyroxine Sodium IP. This experiment was continued for a period of 30 days. In the test, blood was collected on 12th and 30th day through retro-orbital puncture under anesthetic conditions of thiopental sodium 40 mg/kg and separated the serum. By using Chemilumniscent microparticle immune assay method serum concentrations of T3, T4 and TSH were analyzed.

Table 2: Animal Groups

Group No.	Number of Animals required	Route of Administration	Drug given	Period of treatment (days)	Dose
1	6 Animals	Oral	Normal saline	30	-
2	6 Animals	Oral	Methimazole Self made tablet formulation of *Bauhinia Variegata*	0-12 13- 30	10 mg/kg/day 100 mg/kg/day
3	6 Animals	Oral	Methimazole Standard Thyroxine Sodium	0-12 13- 30	10 mg/kg/day 12.5 mg/kg/day

Total Number of Rats required for Protocol = 18

RESULTS AND DISCUSSION

In present study the tablet formulation was evaluated along with HPTLC determination which revealed the following results. In process checks were performed for % moisture content, bulk density, tapped density, hausner's ratio and carr's index were found to be with in standard ranges (Table 3). Results of evaluated uncoated tablet were given in (Table 4) and coated tablet results were given in (Table 5). HPTLC profile of the extracts and tablet formulation are depicted in figure no. 1 and 2 indicate the presence of various spots with different colour at different R_f values. R_f values and colour of the bands from separated compounds are recorded in (Table 6, 7). Test for specific microorganism, Total microbial plate count (TMC) and Total yeast and mould count (YMC) results were found to be within standard ranges (Table 8). Tablets were evaluated for determining its effects in the regulation of hypothyroidism using Albino

wistar rat model. Serum triiodothyronine, thyroxine and thyroid stimulating hormone concentrations were tested as the end parameters of thyroid function in the study. On 12th day both the two groups except normal control group of methimazole induced showed lowered T3 and T4 value and elevated TSH value. After 30 days administration of tablet, lowered the T3 and T4 level and elevated TSH level reversed to that of normal and showed a comparable activity to that of Thyroxine sodium standard used (Table 9, 10). The results showed that the activity of the prepared tablet formulations significantly increase T3 and T4 level and reduced TSH level.

Table 3: Evaluation of Granules (In process check)

Test	Batch 01	Batch 02	Batch 03
% moisture content	4.81	4.99	4.35
Angle of repose	14.8261 (Excellent flow)	17.68 (Excellent flow)	15.94 (Excellent flow)
Bulk density (g/ml)	0.44	0.43	0.42
Tapped density (g/ml)	0.55	0.52	0.51
Carr's Index (%)	20 (Fair to passable)	17.30 (Good flow)	17.64 (Good flow)
Hausner's ratio	1.25 (Good flow)	1.209 (Good flow)	1.214 (Good flow)

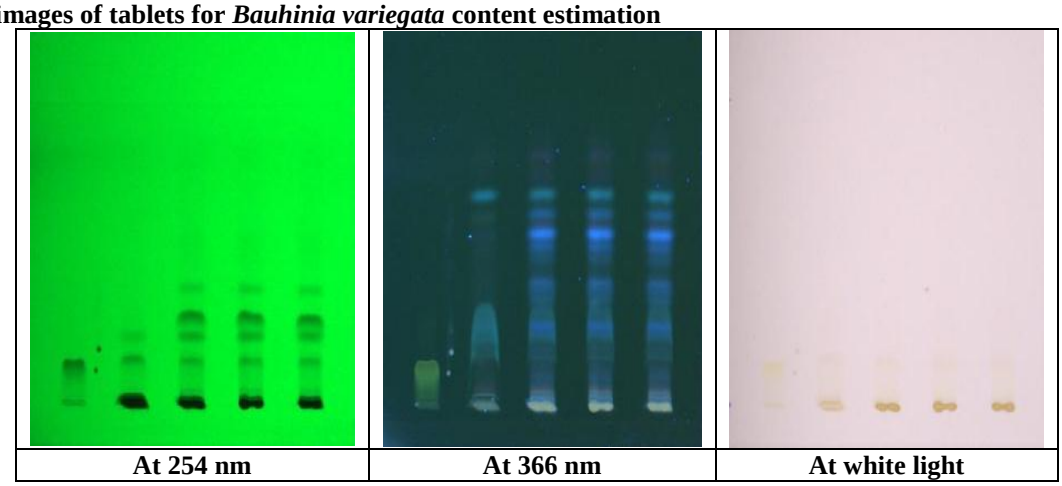
Table 4: Evaluation of uncoated tablet

Test	Batch 01	Batch 02	Batch 03
Friability (%)	0.06976	0.01415	0.02243
Weight variation (gm)	0.792795	0.84581	0.84194
Hardness (kg/cm^2)	6.48	5.38	6.55
Length (mm)	19.312	19.39	19.34
Width (mm)	8.397	8.243	8.281
Thickness (mm)	5.5264	5.555	5.503
Disintegration Time (min)	13.39	13.95	14.93

Table 5: Evaluation of coated tablet

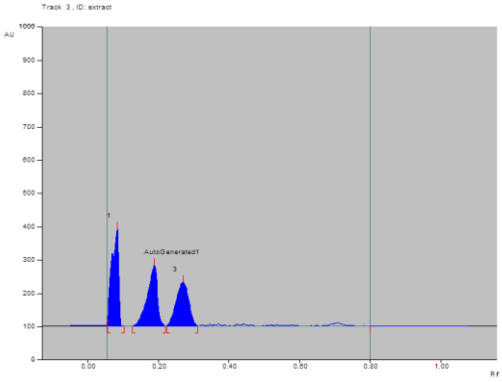
Test	Batch 01	Batch 02	Batch 03
Weight variation (gm)	0.84953	0.8581	0.8592
Hardness (kg/cm^2)	7.525	5.8	7.00
Length (mm)	19.416	19.51	19.54
Width (mm)	8.349	8.366	8.431
Thickness (mm)	5.554	5.650	5.630
Disintegration Time (min)	17.06	22.35	18.20

HPTLC images of tablets for *Bauhinia variegata* content estimation

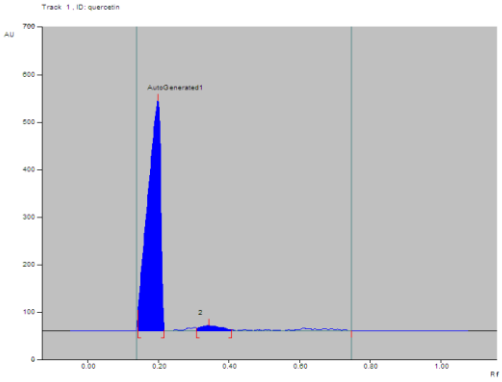


At 254 nm | **At 366 nm** | **At white light**

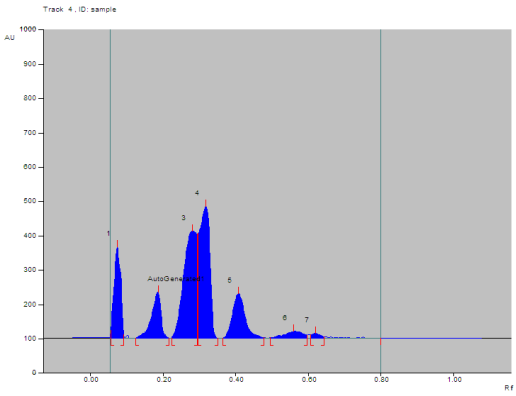
Fig. 1: HPTLC images of *Bauhinia variegata* content estimation.



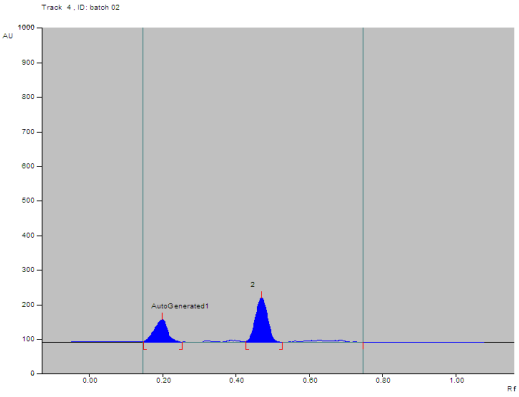
Graph 1: Quercetin standard.



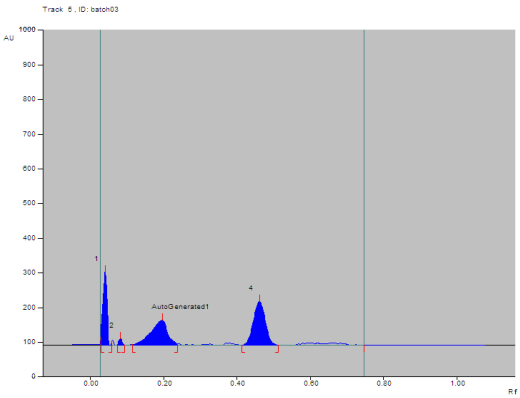
Graph 2: Kanchanar extract.



Graph 3: Batch 01 (Tablet formulation).



Graph 4: Batch 02 (Tablet formulation).



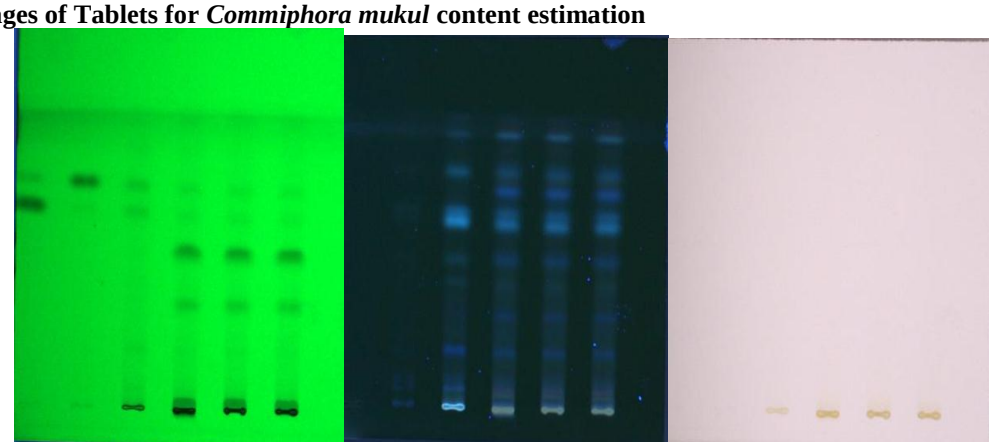
Graph 5: Batch 03 (Tablet formulation).

Table 6: Peak list and R_f value of the chromatogram of 10 µl of methanol extract for *Bauhinia variegate* content estimation

Track	Peak	Max. position (R_f)	Max. height (AU)	Area (AU)
1	1	0.20	484.8	12611.4
1	2	0.34	11.1	480.6
2	1	0.21	103.6	2847.6
3	1	0.20	68.3	1856.6
3	2	0.48	124.0	2914.0
4	1	0.20	68.1	1929.7
4	2	0.47	128.2	3041.1
5	1	0.20	65.0	1838.2
5	2	0.46	125.8	2929.1

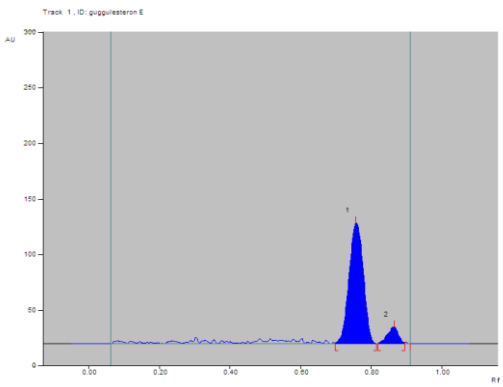
[Track 1: Quercetin standard, Track 2: Kanchanar extract, Track 3: Batch 01 (Tablet formulation), Track 4: Batch 02 (Tablet formulation), Track 5: Batch 03 (Tablet formulation)].

HPTLC images of Tablets for *Commiphora mukul* content estimation

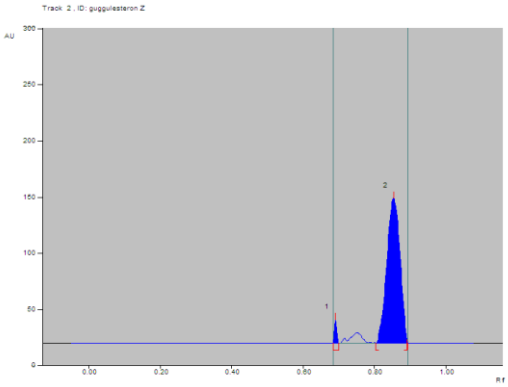


At 254 nm **At 366 nm** **At white light**

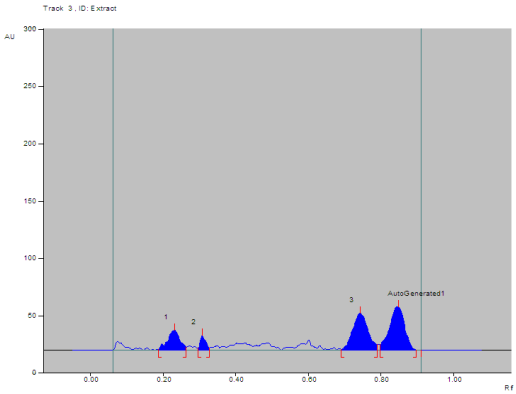
Fig 2: HPTLC images of *Commiphora mukul* content estimation.



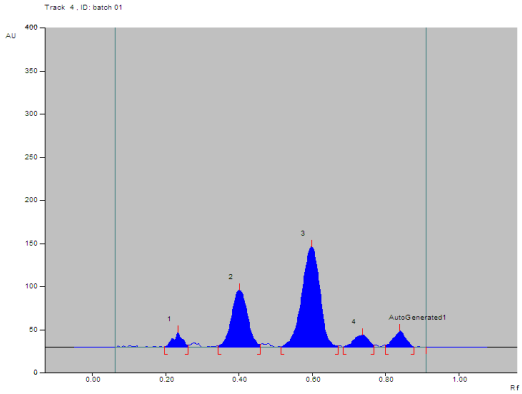
Graph 1: Guggulsterone E standard.



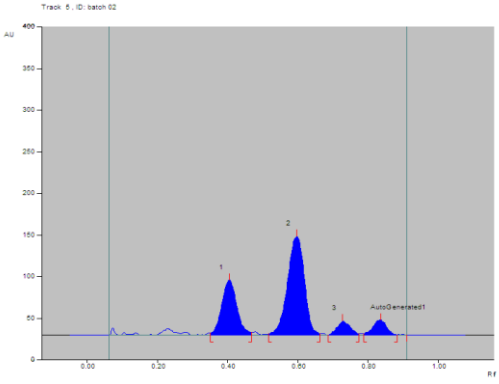
Graph 2: Guggulsterone Z standard.



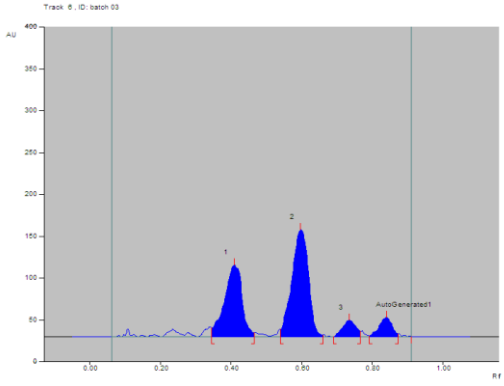
Graph 3: Guggul extract.



Graph 4: Batch 01 (Tablet formulation).



Graph 5: Batch 02 (Tablet formulation).



Graph 6: Batch 03 (Tablet formulation).

Table 7: Peak list and R_f value of the chromatogram of 10 µl of methanol extract for *Commiphora mukul* content estimation

Track	Peak	Max. position (R_f)	Max. height (AU)	Area (AU)
1	1	0.76	101.4	2908.5
1	2	0.86	14.2	319.1
2	1	0.85	126.8	3477.0
3	1	0.74	25.0	705.5
3	2	0.85	34.5	1012.0
4	1	0.84	17.1	414.5
5	1	0.83	15.9	437.3
6	1	0.73	12.7	305.4
6	2	0.84	20.6	540.7

[Track 1: Guggulsterone E standard, Track 2: Guggulsterone Z standard, Track 3: Guggul extract, Track 4: Batch 01 (Tablet formulation), Track 5: Batch 02 (Tablet formulation), Track 6: Batch 03 (Tablet formulation)].

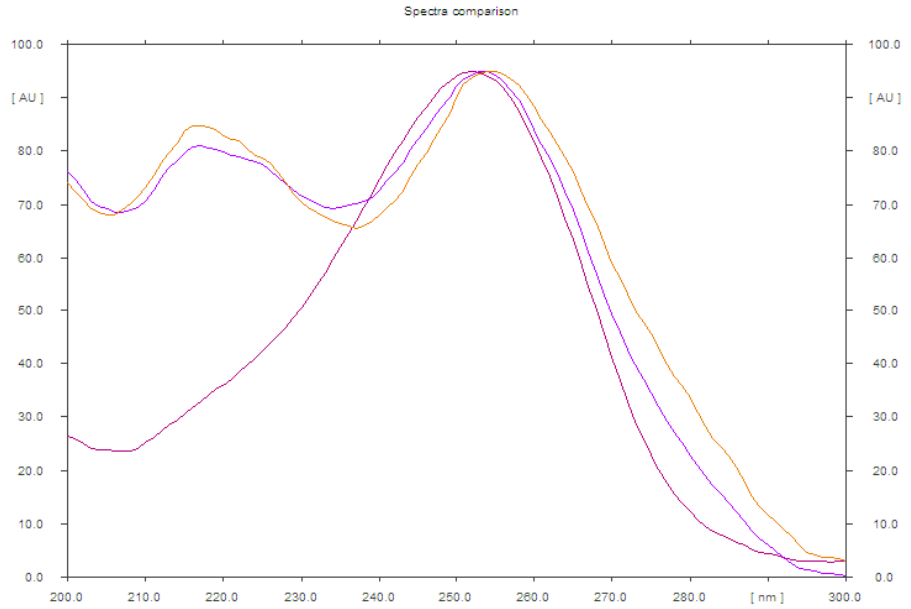


Fig. 3: Spectra overlay.

Table 8: Microbial test

Sr. No.	Parameters	Acceptance criteria	Results Batch 01	Results Batch 02	Results Batch 03
1	*Staphylococcus aureus*	Absent/g	Absent	Absent	Absent
2	*Salmonella species*	Absent/g	Absent	Absent	Absent
3	*Pseudomonas aeruginosa*	Absent/g	Absent	Absent	Absent
4	*Escherichia Coli*	Absent/g	Absent	Absent	Absent
5	Total microbial plate count (TMC)	NMT 10^5 cfu/g	1000 cfu/g	700 cfu/g	850 cfu/g
6	Total yeast and mould count (YMC)	NMT 10^3 cfu/g	10 cfu/g	10 cfu/g	< 10 cfu/g

Table 9: Values of thyroid function test (TFT) on day 12 (Induction of hypothyroidism)

Groups	TSH value (IU/mL)	T3 value (ng/dL)	T4 value (µg/dL)
Normal control	0.520	74.5	1.727
Test tablet formulation	0.600	70.2	1.53
Thyroxine sodium treated standard	0.591	71.5	1.64

Values are expressed as mean, n = 6 per group

Table 10: Effect of tablet formulation on values of thyroid function test (TFT) on day 30

Groups	TSH value (IU/mL)	T3 value (ng/dL)	T4 value (µg/dL)
Normal control	0.521	74.5	1.70
Test tablet formulation	0.531	76.6	1.93
Thyroxine sodium treated standard	0.426	80.7	2.10

Values are expressed as mean, n = 6 per group

CONCLUSION

The developed polyherbal tablets may find its applications as thyroid supplement which can be given easily and safely to patients suffering from thyroid disorders and overcome the symptoms associated with thyroid disorders.

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