

To Identify the Activity Anti hypothyroidism of the Petroleum Ether Extract Phytoconstituents “Phyllanthin and Hypophyllanthin” Isolated from *Phyllanthus Amarus* Linn.

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Submitted: 20-10-2023

Accepted: 30-10-2023

ABSTRACT:

Hypothyroidism is associated with a deficiency in the production of thyroid hormones viz. T₃ and T₄ which is common in hilly areas and all around the world. The problem of iodine deficiency disorders (IDD) is hypothesized to be aggravated by malnutrition, environmental goitrogens and microbial pollution in these areas. Worldwide, thyroid dysfunction is a probable risk factor for 1 to 1.5 billion people usually considered due to simple iodine deficiency and presented as goiter (at least 200 million people). Amongst the various thyroid disorders such as hypothyroidism and hyperthyroidism; hypothyroidism is more common. Women are more prone to thyroid disorders especially those having a history of autoimmune disorders.

Keywords: Hypothyroidism, phyllanthin, hypophyllanthin, T₃, T₄, TSH, nanoparticle phytosomes,

I. INTRODUCTION:

In the present studies, the curative activity of Phyllanthin, Hypophyllanthin, a combination of Phyllanthin and Hypophyllanthin as well as of 50% Hydro-alcoholic extract of *Phyllanthus amarus* were studied and the results were compared with the marketed drug Levothyroxine (Eltroxin) using the model of Propylthiouracil induced hypothyroidism in Wistar albino rats for a period of 22 days(1,2). A study was also carried out to elucidate the TNF-alpha levels in order to evaluate the probable mechanism of action. Hypothyroidism is conventionally treated by administering a daily dose of synthetic thyroid hormones e.g. Levothyroxine(3,4). However one of the most important adverse effects associated with synthetic thyroid hormone therapy is hyperthyroidism, A higher dose could cause symptoms of nervousness, palpitations or insomnia, typical of hyperthyroidism(5,6).

Herbal medicines are now in great demand in the developing world for primary health care for

better cultural acceptability, better compatibility with the human body and minimal side effects. So far, no drug is available for the treatment of hypothyroidism or hyperthyroidism preventive or curative for human use in any system of medicine all over the world(7,8). *Phyllanthus amarus* (Family: Euphorbiaceae) henceforth referred to as PA has been reported to possess thyroxine-like activity. Work was done to explore the thyroxine-like activity of Phyllanthin, Hypophyllanthin and a combination of both against Propylthiouracil hypothyroidism and it was concluded that phyllanthin and hypophyllanthin possess the thyroxine-like activity and two compounds when administered together were found to possess more activity(9,10).

❖ Hypothyroidism

Comparison of studies of the prevalence and incidence of hypothyroidism is hampered by differing definitions and population samples. According to Anita McGrogan (2008), the reported incidence of autoimmune hypothyroidism varied between 2.2/100 000/year (males) and 498.4/100 000/year (females) and for autoimmune hyperthyroidism, incidence ranged from 0.70/100 000/year (Black males) to 99/100 000/year (Caucasian females)(11,12). Higher incidence rates were found in women compared to men for all types of autoimmune thyroid disease. Their best estimates of the incidence of hypothyroidism are 350/100 000/year in women and 80/100 000/year in men; the incidence of hyperthyroidism is 80/100 000/year in women and 8/100 000/year in men according to Bilous RW, Tunbridge WM(1988) the annual incidence of overt hypothyroidism is between 1-2/1000 for female and around 2/10,000 for males, with individuals having previously elevated TSH and positive circulating thyroid autoantibodies being particularly at a risk(12).

II. MATERIALS AND METHODS

The authenticated seeds of the plant PA were obtained from Central Institute for Medicinal and Aromatic Plants (CMAP, Lucknow, India). Further in order to get authentic and uniform samples of PA the seeds were sown in the DIPSAR Herbal Garden in April. The field was kept absolutely free from weeds for which regular hand weeding was done in every 15 days. The plant was harvested in two phases. The first phase of collection was done in September and the next phase was done in October. The plant was dried for a duration of 15 days at $25\pm 5^{\circ}\text{C}$ in shade. The leaves of the plant were separated and subjected to size reduction using sieves of mesh sizes 60 and 120. From the complete dried leaves of PA phyllanthin and hypophyllanthin were extracted using petroleum ether (60°C - 80°C), the petroleum ether extract obtained was then concentrated under reduced pressure to obtain a dry extract (yield 94.79%). From this dry extract, 1.5267 gm of phyllanthin (flakes) was isolated and crystallised from a mixture of diethyl ether: petroleum ether (1:10) and 505.2 mg of hypophyllanthin (prisms) was isolated and crystallised from alcohol. The isolated phyllanthin and hypophyllanthin were identified using analytical tools like U.V, TLC, HPTLC, and DSC and were standardized by MASS and FTIR spectroscopy. The isolated compounds were compared with standards, gifted by Natural Remedies, Bangalore. All the values were compared with the reported values.

For preparing the Hydroalcoholic extract the dried and size-reduced leaves of PA were extracted with 50% ethanol-water in the proportion of 1:3, being stirred and macerated at room temperature ($21\pm 3^{\circ}\text{C}$) for 15 days. The ethanol was removed and the extract was concentrated to the desired level, lyophilized to get powder weighing about 150 gm and stored at -20°C .

III. RESULTS AND CONCLUSION

1. COLLECTION OF CRUDE DRUG.

The crude drug *Phyllanthus amarus* was collected and properly preserved.

2. AUTHENTICATION OF THE *Phyllanthus amarus*.

The crude drug sample was authenticated by *Phyllanthus amarus*.

3. EXTRACTION OF *Phyllanthus amarus*.

From the complete dried leaves (1.0kg) 62.93gm of dry extract was obtained.

The percentage yield of the dried extract was found to be 6.29 %.

4. ISOLATION OF PHYLLANTHIN AND HYPOPHYLLANTHIN.

The yield value of Phyllanthin and Hypophyllanthin were found to be 0.4142% and 0.05% respectively from the crude extract of *Phyllanthus amarus*.

5 CHROMATOGRAPHIC PROFILING OF PHYLLANTHIN AND HYPOPHYLLANTHIN WITH REFERENCE TO STANDARD BY TLC.

The R_f value of Phyllanthin and Hypophyllanthin was found to be 0.36 and 0.39 respectively in isolated compounds. The R_f value of standard Phyllanthin and Hypophyllanthin was found to be 0.37 and 0.38 respectively.

❖ TLC OF ISOLATED PHYLLANTHIN.

R_f value for test Phyllanthin = 0.36

R_f value for standard Phyllanthin = 0.37

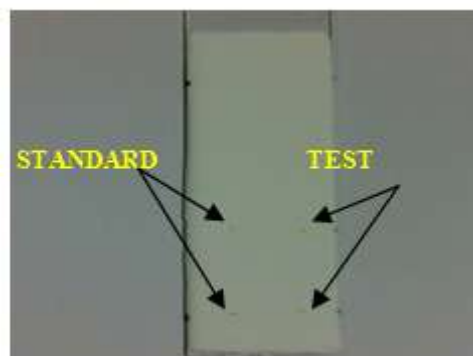


Fig1: TLC of Isolated Phyllanthin.

❖ TLC OF ISOLATED HYPOPHYLLANTHIN.

R_f value for test Hypophyllanthin = 0.39

R_f value for standard Hypophyllanthin = 0.38

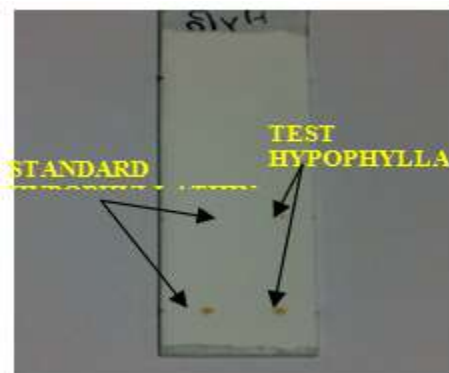


Fig2: TLC of Isolated Hypophyllanthin

6. CONFIRMATION OF PHYLLANTHIN AND HYPOPHYLLANTHIN BY CHEMICAL TESTS.

- Phyllanthin: Concentrated Sulphuric acid was added to the crystals, and pink colour was obtained in a cold solution which turned green and then blue on warming.
- Hypophyllanthin: Concentrated Sulphuric acid was added to the crystals, and an orange colour was obtained in a cold solution which turned bright red on warming.

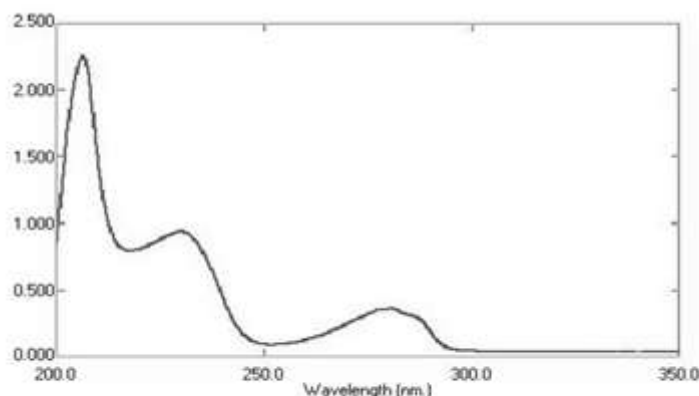
7. CHARACTERIZATION OF PHYLLANTHIN AND HYPOPHYLLANTHIN BY UV, NMR & FTIR WITH REFERENCE TO STANDARDS.

7.1 UV Spectroscopy of the isolated Phyllanthin, Hypophyllanthin and standards The absorbance of Phyllanthin and Hypophyllanthin was found to be 280nm and 278nm respectively. This is similar to the standards UV SPECTROSCOPY OF TEST PHYLLANTHIN.

Concentration of Phyllanthin in UV-Absorption of 0.0192% w/v solution in methanol

S. No	wavelength(nm)	Abs
1	280	0.354
2	229	0.965
3	206	2.194

Table1: UV of test Phyllanthin.



UV - Absorption of 0.0192% w/v solution in methanol

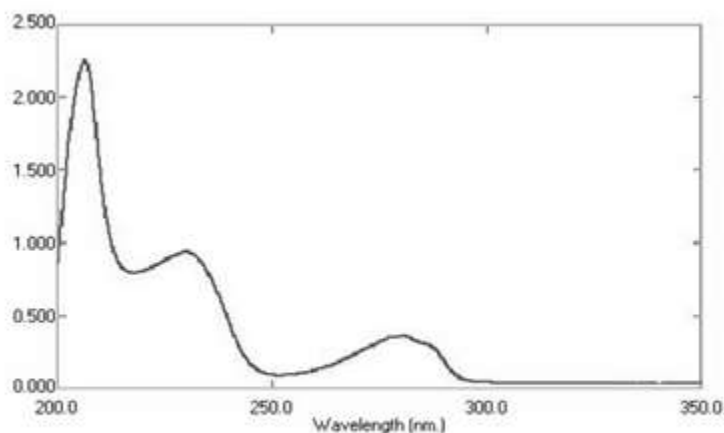
Fig3: UV spectroscopy of test phyllanthin

UV SPECTROSCOPY OF STANDARD PHYLLANTHIN.

Concentration of Phyllanthin in UV-Absorption of 0.0192% w/v solution in methanol

S. No	wave length(nm)	Abs
1	280	0.354
2	229	0.965
3	206	2.194

Table2: UV of standard Phyllanthin



UV - Absorption of 0.0192% w/v solution in methanol

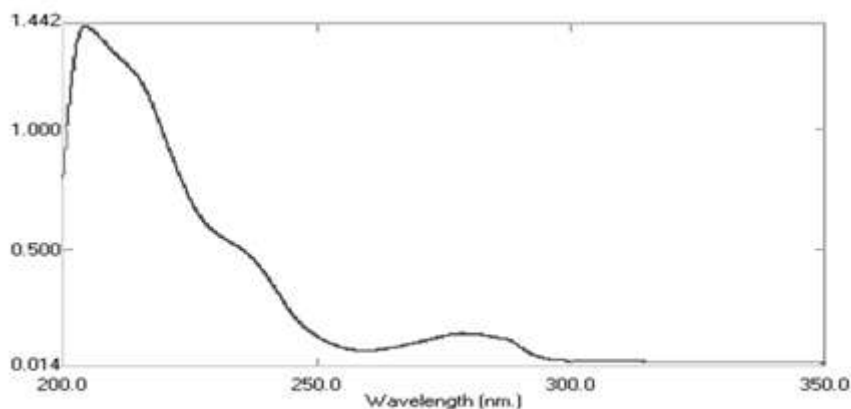
Fig4: UV Spectroscopy of Standard Phyllanthin

UV SPECTROSCOPY OF TEST HYPOPHYLLANTHIN

Concentration of Hypophyllanthin in UV –Absorption of 0.0184 % w/v solution in methanol.

S. No.	Wave length (nm)	Abs
1	278	0.226
2	210	2.360

Table.3: UV of test Hypophyllanthin



UV - Absorption of 0.0184% w/v solution in methanol

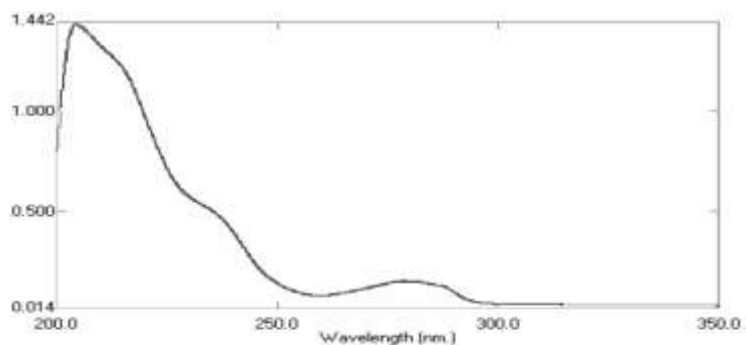
Fig.5: UV Spectroscopy OF Test Hypophyllanthin

UV SPECTROSCOPY OF STANDARD HYPOPHYLLANTHIN

Concentration of Hypophyllanthin in UV –Absorption of 0.0184 % w/v solution in methanol.

S. No.	Wavelength (nm)	Abs
1	278	0.226
2	210	2.360

Table.4: UV of standard Hypophyllanthin



UV - Absorption of 0.0184% w/v solution in methanol

Fig.6: - UV Spectroscopy of Standard Hypophyllanthin

7.2 Characterization of Phyllanthin and Hypophyllanthin by NMR

It was found that the NMR part of Phyllanthin and Hypophyllanthin was found to be similar with respect to the standards.

NMR OF TEST PHYLLANTHIN

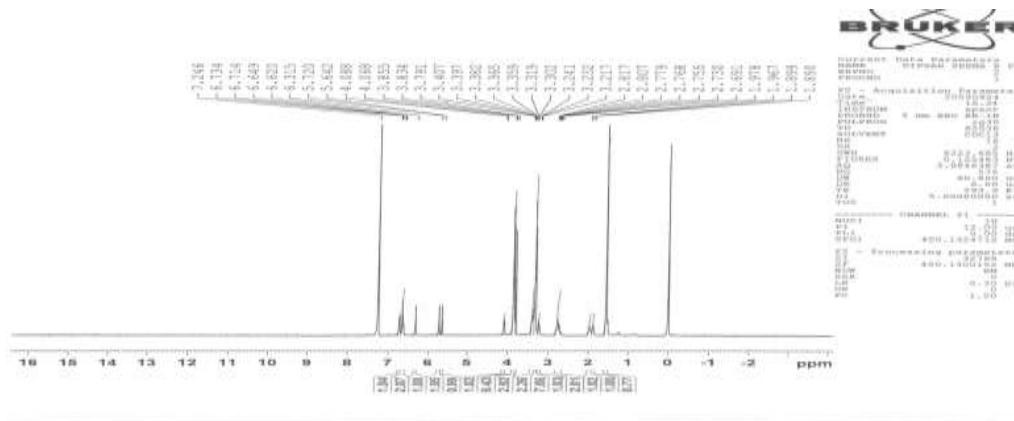


Fig.7: NMR of Test Phyllanthin

NMR OF STANDARD PHYLLANTHIN

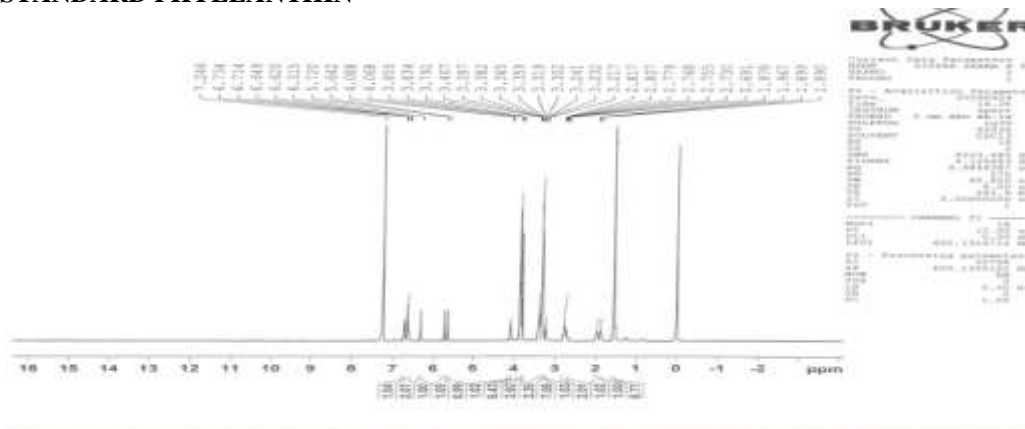
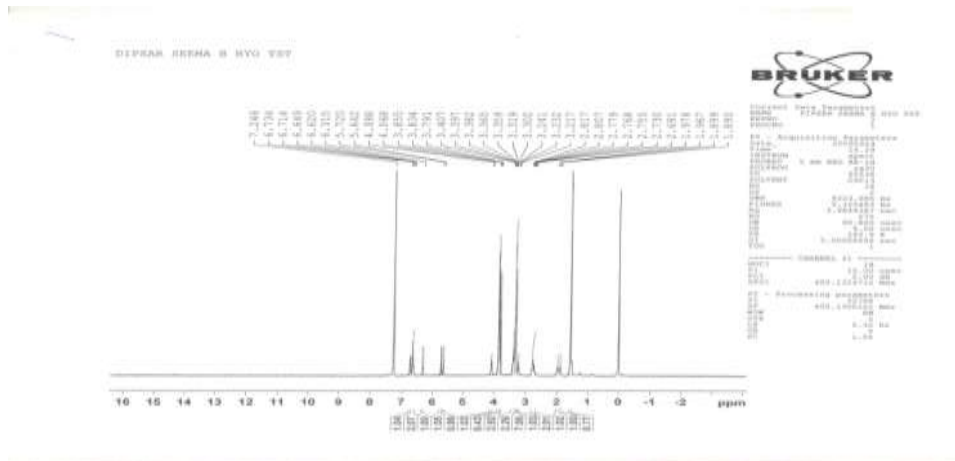


Fig.8: NMR of Standard Phyllanthin

NMR OF TEST HYPOPHYLLANTHIN



FTIR STANDARD PHYLLANTHIN



Fig.11: FTIR of Phyllanthin Standard

FTIR OF PHYLLANTHIN SAMPLE

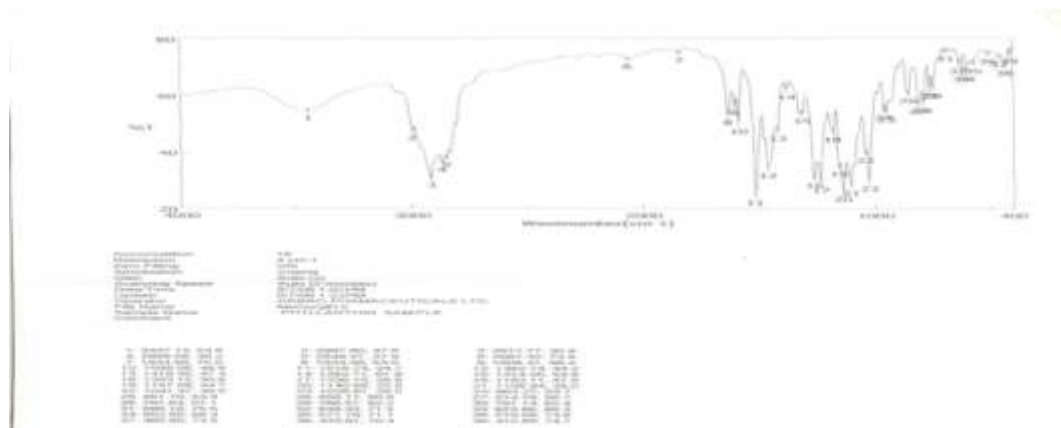


Fig.12: FTIR of Phyllanthin Sample

FTIR OF HYPOPHYLLANTHIN STANDARD



Fig.13: FTIR of Hypophyllanthin standard

FTIR OF HYPOPHYLLANTHIN SAMPLE



Fig.14: FTIR of Hypophyllanthin Sample

8. PREPARATION OF PHYTOSOMES OF PHYLLANTHIN AND HYPOPHYLLANTHIN

8.1 Preparation of phyllanthin and hypophyllanthin-phospholipid complex

Prepared Phyllanthin and Hypophyllanthin–phospholipid complex according to different quantity ratios of phospholipids and drugs, such as 1mg, 3mg, and 6mg quantity of drugs. The results showed that when the ratio was more than 1, the appearance of resultant materials appeared viscous and it was not easy for resultant materials to be prepared for other preparations, but when the ratio was lower than 1, the stability of phospholipid complexes was worse. For the purpose of getting the best quality and using the fewest quantity of Phospholipids, at last, we prepared Phyllanthin and Hypophyllanthin–phospholipid complex in terms of the quantity ratio 1.

9. CHARACTERIZATION OF THE PREPARED PHYTOSOMES OF PHYLLANTHIN AND HYPOPHYLLANTHIN

9.1 Particle size measurement

The Phytosomes formulations obtained using chloroform and methanol (7:3) have similar physiochemical properties like size and shape. Multilamellar vesicles (MLVs) were obtained as a result of thin film hydration. Optical microscopy confirms the formation of MLVs of similar morphology in both formulations. Evaporation under vacuum resulted in the formation of unilamellar vesicles of MLVs with a particle size of 120nm (75 to 500nm) determined by ZEN3600 Zetasizer (MALVERN INSTRUMENTS, UK).

ACKNOWLEDGEMENT

Authors would like to thank, Sunrise University, Bagad Rajput, Tehsil-Ramgarh, Alwar Rajasthan, India for extending their facilities.

CONFLICT OF INTEREST

The study's authors certify that they have no known financial or personal conflicts of interest that could skew the results.

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