e-ISSN: 0975-5160, p-ISSN: 2820-2651

Available online on www.ijtpr.com

International Journal of Toxicological and Pharmacological Research 2024; 14(11); 74-80

Original Research Article

Spectrophotometry – A Tool to Assess Corticosteroids and Tectona Grandis

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Received: 18-08-2024 / Revised: 21-09-2024 / Accepted: 26-10-2024
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Conflict of interest: Nil

Abstract:

Background: Glucocorticoids- Prednisolone, Hydrocortisone are available in various formulations like oral and parenteral, inhalation and topical. Though they are many medicinal benefits of steroids nowadays, this study aims to compare the natural product Tectona grandis which possess steroid like activity with the Standard steroids like Hydrocortisone Aim of our study is to determine the potency of Tectona grandis decoction with Standard steroids (Prednisolone, Hydrocortisone) by analytical method and to compare steroid in Tectona grandis extract with Standard Steroid by FTIR and HPTLC.

Methodology: 20 mg of Tectona grandis's bark were dried, weighted and made in to fine powder using blender, thus Tectona grandis powder was prepared. The processed Tectona grandis were fractionated /extracted using chloroform. The extract were subjected to phytochemical analysis focusing on phytosterols by two methods- Salkowski test and Lieberman burchardt test. Estimation of Steroid in Tectona grandise as done by Spectrophotometry and HPTLC.

Results: The appearance of reddish brown precipitate by Salkowski test and reddish brown ring formation by Lieberman-burchardt test indicate the presence of phytosterol. TG decoction is equal to 1.25 mg of hydrocortisone and 1.75mg of prednisolone. Tectona grandis extract showed that the spectrum similar to spectrum of Prednisolone whose characteristic band of the OH group were found at 3200-3500 cm-1 and two carbonyl stretching peak appear as a very strong band at 1708cm-1 and 1654 cm-1, denotes Prednisolone like structure in FTIR.

Conclusion: Tectona grandis's stem bark is a commercially available plant product that possess steroidal activity and the structure resembles prednisolone. It can be used to reduce inflammation whenever corticosteroid are indicated as alternative cost effective treatment.

Keywords: Tectona Grandis, Steroid, FTIR, HPTLC.

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Introduction

Corticosteroids like Glucocorticoids and mineralocorticoids are released by the adrenal cortex [1]. Mineralocorticoids acts by retaining sodium and depleting potassium ions. Aldosterone is the most important endocrine mineralocorticoid. Glucocorticoid has an important role in the metabolism of carbohydrates, proteins and calcium and has also an effective anti-inflammatory and immunosuppressive activity. Hydrocortisone, also called cortisol, is the main endogenous glucocorticoid. The estimated physiological production of cortisol is approximately 5 to 10mg/m^2 per day and may increase 5 to 10 times under stress conditions.[2–5]. The usage of corticosteroids in 1949 demonstrates the beneficial effects of adrenocorticotropic hormone in the treatment of allergic diseases. Glucocorticoids- Prednisolone, Hydrocortisone are available in various formulations like oral and parenteral, inhalation and topical [6]. Though they are many medicinal benefits of steroids nowadays, this study aims to compare the natural product Tectona grandis which possess steroid like activity with the Standard steroids like Hydrocortisone, Prednisolone by various analytical method like Smart specs Spectrophotometry, and structure of phytosteroid by FTIR (Fourier transformer Infrared Spectrophotometry) and analysis of Rf (Retardation Factor) value by HPTLC (High performance Thin layer Chromatography). Tectona grandis Linn (Teak), is locally known as Sagwan, belongs to Lamiaceae family which is protective against

fungal infection. Teak has worldwide reputation for its physical and mechanical properties, particularly elasticity, strength, durability decay resistance medicinal properties and traditional uses. All parts of the teak tree has medicinal properties.

The decoction of bark is used in bronchitis, hyperacidity, dysentery, verminosis, burning sensation, diabetes, difficult labour, leprosy and skin diseases(7), treatment of urinary system-related troubles like anuria [8]The methanolic and ethanolic extract of Tectona grandis has wound healing activities. Quinones are present in Tectona grandis in the form of naphthoquinones and anthraquinones contains phytochemicals such as terpenoids, apocarotenoids, phenolic compounds and flavonoids, steroids/saponins, phenyl propanoids (lignans and norlignans) & phenylethanoid glycosides, some fatty esters and miscellaneous compounds [9,10]. Anti-inflammatory and analgesic effect is due to the presence of Steroid like activity and inhibition of Prostaglandin synthesis and this study confirms the presence of Steroid in Tectona grandis decoction and identification of steroid content and its structure in Tectona grandis with respect to standard steroids (Prednisolone and Hydrocortisone, Methyl prednisolone).

Aim of our study is to determine the potency of Tectona grandis decoction with Standard steroids (Prednisolone, Hydrocortisone) by analytical method and to compare steroid in Tectona grandis extract with Standard Steroid by. So to compare the potency of Tectona grandis decoction with standard steroid drug, Also to Evaluate percentage of steroid present in the Tectona grandis in comparison with Standard steroids (Prednisolone and Hydrocortisone, Methyl prednisolone) and to detect and compare steroidal structure in Tectona grandis decoction and extract with Standard Steroids by FTIR (Fourier transformer infrared spectrophotometry) and to analysis Rf value of Tectona grandis by HPTLC (High performance Thin layer chromatography). This is a laboratory based Prospective analytical study which was conducted in Experimental pharmacology lab, Department of pharmacology, Tertiary care hospital, Coimbatore for duration of 1 month.

Methodology:

After getting the IHEC approval, 20 mg of Tectona grandis‘s bark were dried, weighted and made in to fine powder using blender, thus Tectona grandis powder was prepared.

The processed Tectona grandis were fractionated /extracted using chloroform. The extract were subjected to phytochemical analysis focusing on phytosterols by two methods-Salkowski test and Lieberman burchardt test **[**11]

Methods:

Salkowski test: To the chloroform extract of Tectonagrandis, 0.5ml of concentrated sulphuric acid was added and observation were recorded.

Lieberman burchardt test: To chloroform extract of Tectonagrandis few drops of acetic anhydride solution was added followed by adding concentrated sulphuric acid along the sides of the test tube and observation were recorded

II. Estimation of Steroid in Tectonagrandis with steroids by Spectrophotometry-qualitative method to measure steroids:

Preparation of Tectona grandis decoction:

a. 20mg of Tectona grandis powder was mixed with 50ml of Distilled water and boiled to 100˚c for 15 min to obtain decoction and extract were prepared by Soxhlet method [12].
b. An accurately weighted 20mg of standard steroids were made into fine powder and was dissolved in 10 ml of Methanol.
c. 20 mg of Tectona grandis decoction were dissolved in 10ml of Methanol separately. Then the solution were filtered and then washed by adding again 10ml of methanol.
d. All the solutions (Prednisolone, Hydrocortisone, Tectona grandis decoction) were transferred into 10ml conical flask separately and make up with distilled water, to prepare working standard.
e. Five serial dilutions were made in test tube for Standard steroids and Tectona grandis decoction.
f. To each test tube, 0.5ml of combination of three reagents (Sulphuric acid (4N) 2ml, ferric chloride (0.5%) 2ml and potassium ferric cyanide (0.5%) were added. Then this mixture was heated at 70˚c for 30 minutes after shaking the mixture in vortex and absorbance values were measured at 780nm against reagent blank with the help of Smart spec‘s Spectrophotometer(BIORAD) [13]

III. FTIR: Steroid Like Structure: Fourier transform infrared spectrophotometer (FTIR) is the most powerful tools for the identification of types of chemical bonds (functional groups) present in unknown plant compounds. Dried powders of different solvent extracts of plant material were used for FTIR analysis. 10mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample disc. The powdered sample of each plant specimen was loaded in FTIR Spectroscope, with a scan range from 400 to 4000 cm-1 with a resolution of 4cm-1 [14]

IV. HPTLC Fingerprinting Analysis: HPTLC is an reliable, reproducible and standardised method to determine major active constituents in the extract

of Tectona grandis which was spotted using Linomat V applicator in CAMAG HPTLC instrument in Silica gel 60F 254 aluminium precoated plates. The mobile phase used were Chloroform: methanol (8:2) for the ascending development. The resultant plates were derivatized through UV chamber at 254nm and 366nm. The developed chromatogram were scanned using the Wincats software 3. The resultant Rf value of the extract and comparative steroidal moiety was recorded [15]

Results

The appearance of reddish brown precipitate by Salkowski test and reddish brown ring formation by Lieberman-burchardt test indicate the presence of phytosterol.

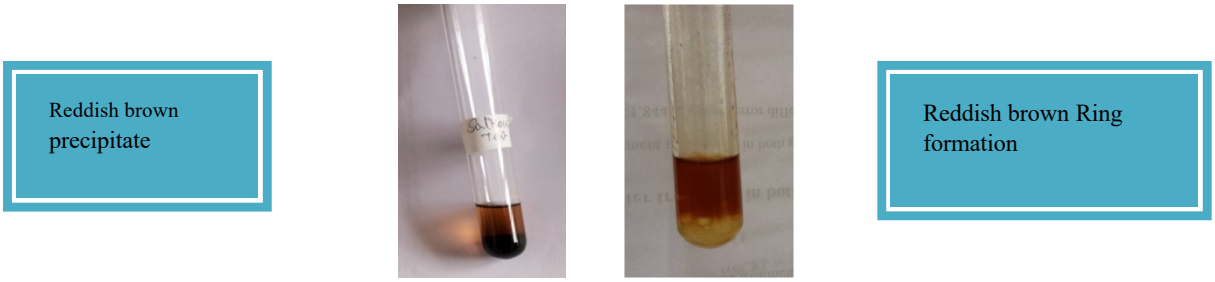

Figure 1: Phytochemical analysis: Salkowski test Lieberman burchardt test

0.2mg of TG powder is equal to 0.125mg of Std Hydrocortisone, 0.175mg of Prednisolone and not comparable with methyl prednisolone. 0.2mg TG decoction is equal to 1.25 mg of hydrocortisone and 1.75mg of prednisolone.

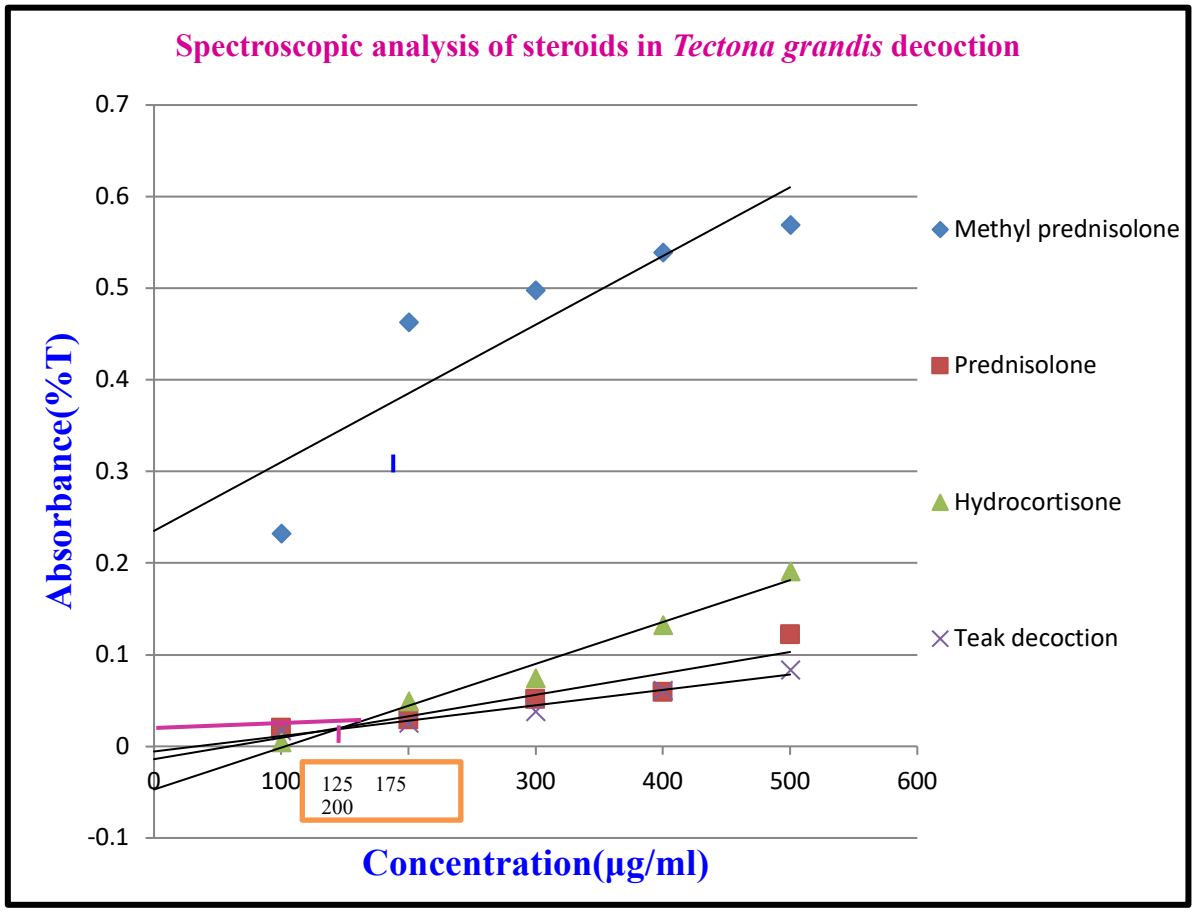

Figure 2: Comparison of Tectona grandis with steroids using spectrophotometry

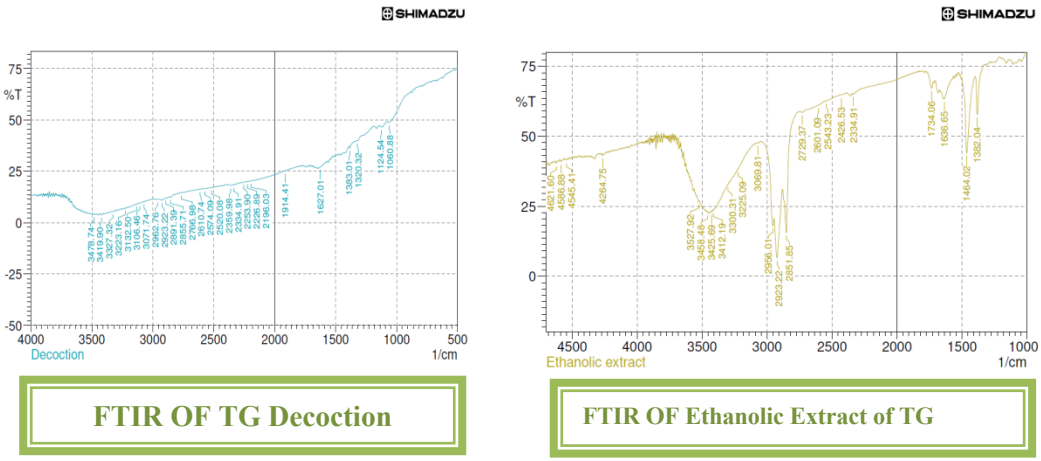

Figure 3: FTIR: Fourier's transform infrared spectrophotometer

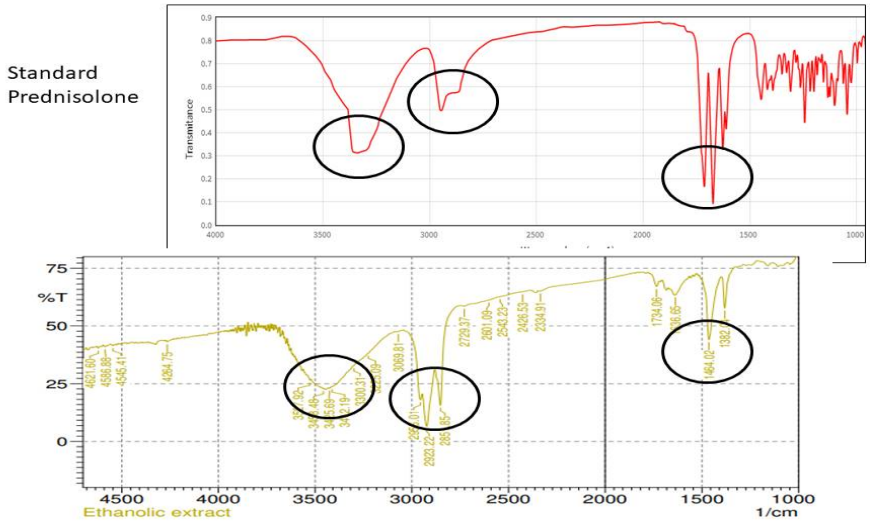

Figure 4: Comparison of FTIR pattern of standard steroid (prednisolone with ethanolic extract)

Tectona grandis extract showed that the spectrum similar to spectrum of Prednisolone whose characteristic band of the OH group were found at 3200-3500 cm-1 and two carbonyl stretching peak appear as a very strong band at 1708cm-1 and 1654 cm-1[5], denotes Prednisolone like structure.

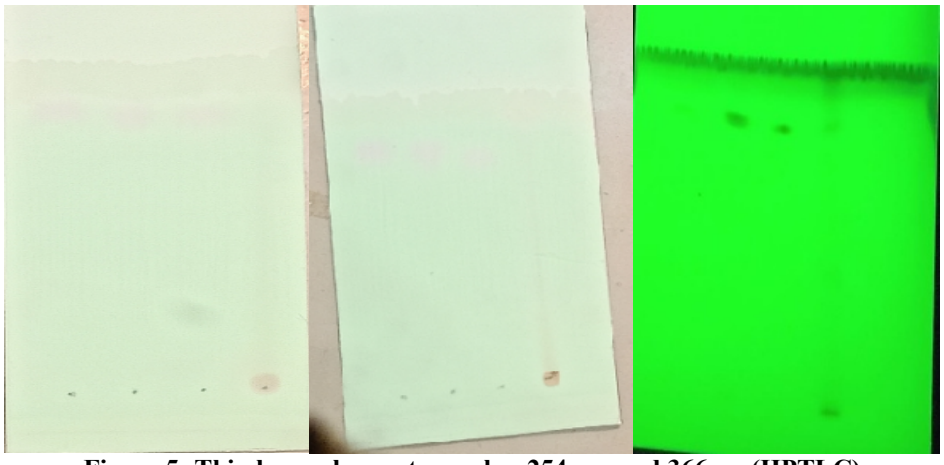

Figure 5: Thin layer chromatography: 254 nm and 366nm (HPTLC)

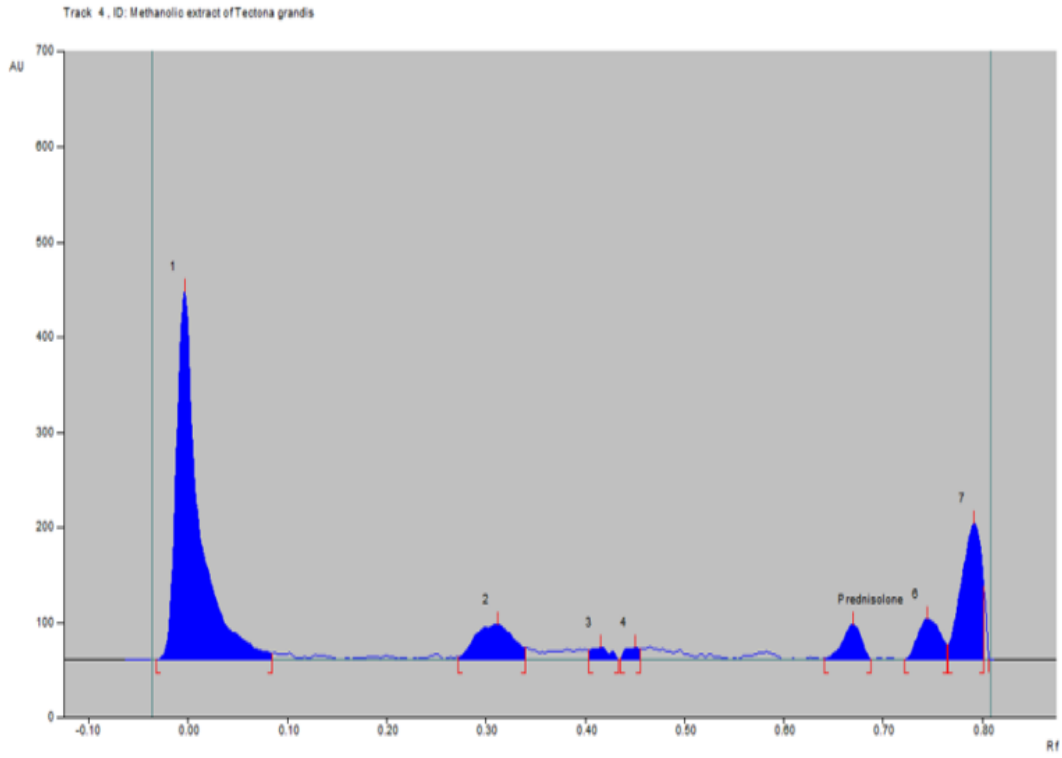

Figure 6: Chromatogram for Tectona grandis Extract

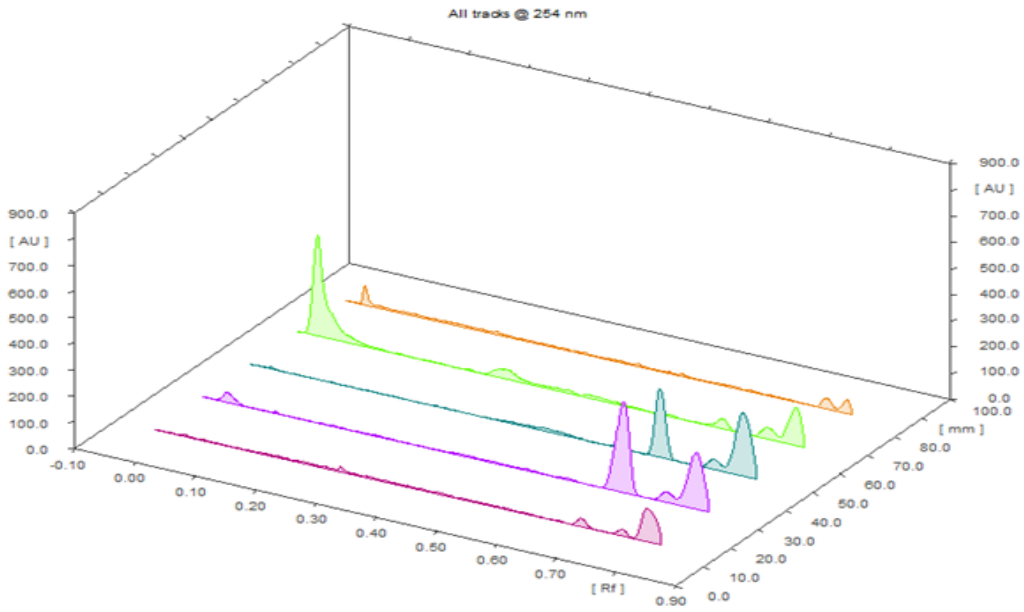

Figure 7: 3D Chromatogram for Standard and Tectona grandis extract

Table 1: Fingerprinting profile of Tectona grandis extract using HPTLC

S.no	Particulars	Report
1.	Solvent system	Chloroform: Ethyl acetate(8:2)
2	Standard Rf(Prednisolone)	**0.67**
3.	Standard Rf(Methyl Prednisolone)	0.65
4.	Standard Rf(Hydrocortisone)	0.66
3.	TG Extract with various Rf Value)-presence of **Prednisolone**	0.31,0.45,0.42,0.75,0.79,0.27,0.74,0.00,0.50,**0.67**

Discussion

Corticosteroids acts by two mechanism, one is by binding with specific receptor proteins in target tissues for the expression of responsive genes by altering the types of synthesized proteins .This mechanism of action is known as genomic action, and it depends on protein synthesis, and it does not exert an immediate effect on the target tissue [16].

In addition to the genomic action, the non-genomic immediate effects are associated with the physicochemical interaction with membrane receptors. Glucocorticoids have the ability to bind to receptors for cortisol and trigger a variety of cardiovascular, metabolic, immunological and homeostatic effects. Glucocorticoids reduces cell mediated immunity by inhibiting the genes encoding the production of interleukins (IL) and tumor necrosis factor alpha (TNF-a), especially IL-2, resulting in reduction of proliferation and activation of T cells. Therefore steroids are helpful in various inflammatory condition and at the same time,

Tectona grandis has also found to have medicinal properties like steroid. Therefore this study evolves to confirm the effectiveness and potency of Tectona grandis in both decoction and ethanolic extract by Spectrophotometer and the Structural type of steroid is confirmed by various method like FTIR,TLC and HPTLC (spectrophotometer fig 1and fig 2- Potency of 200µg Teak is equal to 62.5% of hydrocortisone and 87.5% prednisolone(fig.1) .0.2mg of Tectona grandis Extract is equal to 0.125mg of hydrocortisone and 0.175mg (0.2mg) of prednisolone, FTIR concludes that the spectrum of Tectona grandis resembles Prednisolone like structure(Image-3).

Prednisolone paves a way to use this bark powder decoction as a supportive therapy in patient who cannot tolerate steroid in specific clinical condition. Topical decoction also helps a patient with super inflammatory condition including burns in small scale, cost effective, nd self-treatment mode. TLC shows the presence of Steroid (Prednisolone) at 254 and 366nm.HPTLC shows the pattern resembling prednisolone with spectrum of Rf value 0.67.

Conclusion

Tectona grandis stem bark is a commercially available plant product that possess steroidal activity and the structure resembles prednisolone .It can be used to reduce inflammation whenever corticosteroid are indicated as alternative cost effective treatment.

Further studies are required to confirm the above findings.

Acknowledgement: I express my gratitude and sincere thanks to Dr. K. Bhuvaneswari M.D., PGDBE, Professor and Head of Department of Pharmacology, PSG Institute of Medical Sciences & Research, for being my guide. It was her valuable suggestions, guidance and constant encouragement in every steps that has helped me to complete my research work.

I am obliged to thanks Dr. G. Syamala M. Pharm PhD, Department of Pharmacognosy, PSG college of Pharmacy, for guiding me in obtaining result in HPTLC

I express my whole hearted thanks to clinical faculties and postgraduates- Dr. J Asvini, Dr. M. Jegatheeswari for their timely help and support.

I wish to extend my sincere thanks to department Lab technicians (Mrs. A. Maragatham & Mrs. C. Rajammal) and Lab assistants (Mr. C. Ramesh& Mr. G. Padmanaban)

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