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Isolation, Characterisation And Estimation Of β-Lapachone Present In The Tecomaria Capensis By Uv - Spectrophotometric Method

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Abstract: Tecomaria capensis (Bignoniaceae) is otherwise called tecoma. It is an evergreen scrambler widely grows in forest margins but more commonly along drainage lines in dense woodland. It is used for the treatment of fever, pneumonia and stomach troubles also rubbed on bleeding gums to promote blood clotting. We aimed to explore the pharmacognistic and phytochemical screening of leaves of Tecomaria capensis. Separation of lapachone was attempted. The separated lapachone was characterised by HRBC stabilisation method. The study paved the presence of lapachone in Ethylacetate and ethanol extracts of Tecomaria capensis.

Index terms: Phytochemical, β-lapachone, UV-Visible Spectrophotometry.

Introduction:

Tecoma capensis (common name Cape honeysuckle) [1,2,3] is a species of flowering plant in the family Bignoniaceae, native to southern Africa. It is an attractive ornamental garden plant commonly used for screening and decorative purposes, and can also be trimmed to form a hedge. The powdered bark is used in the treatment of fevers, pneumonia and stomach troubles [4, 5]. The powdered bark is rubbed on bleeding gums to promote blood clotting. A leaf decoction is used in the treatment of diarrhoea and for intestinal inflammation [6,7]. It is believed to ease pain and produce sleep.

Materials and methods:

Instruments:

UV-Visible Spectrophotometer (Systronics model 2203) an electronic balance (Shimadzu TX223L), digital p^H meter (Systronics model 1802), and an ultrasonic sonicator (spectra lab, model 40).

Chemicals and reagents:

The lapachone was procured from chloroform, sulphuric acid, ethanol, methanol were purchased from SD- fine and Merck pvt. Ltd. Mumbai, India.

Collection and identification of plant materials:

The leaves of Tecomaria capensis (Thunb) Spach. Were collected from Guntur district, A.P and it was authenticated by professor Dr.S.M.Khasim, Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur. The specimen (No: ANU/00129/2009/AP) was deposited in the department of botany and microbiology for future reference.

Preparation of reagents and standards:

Mobile phase:

To 70 parts of toluene, 30 parts of ethyl acetate was mixed to get one litre of the mobile phase. The mobile phase was then filtered through 0.22 µm nylon membrane vacuum filtration and degassed by sonication.

Preparation of standard solution:

Weigh 0.1gm of β-lapachone standard drug and add 100ml of methanol was added. Take certain concentration and to prepare 0.2, 04, 0.6, 0.8 and 1µg/ml. After that we go for UV spectrophotometry. And it obtain linearity curve.

Preparation of plant extract:

Tecomaria capensis leaves were collected from healthy plant and cleaned, washed with water, remove the needles, dried for 3 days and mechanically powdered for obtained course powder. It is subjected to extraction in a Soxhlet condenser apparatus by methanol. For removing moistures such kg of the leaf powder is soaked in 2 litre petroleum ether for 72 hours by 3 times and the leaf separated and dried. Similarly by benzene the moistures were removed The petroleum ether and benzene extracts does not show the alkaloid presents while performing Mayer's and Dragendorff's reagent alkaloid test. The leaf powder dried completely from benzene and is soaked in methanol for 96 hours. The methanol extract collected in air tight container. The menthol extract prepared for 2 times from the coarse powder. The collective methanol extract filtered multiple times by #1 waterman paper for removing of powder and sediments. A Soxhlet condenser arrangement is used for evaporating the excess methanol

from methanol extract by mantel arrangement at 60 ^{0} C. The methanol evaporates up to its 1/20th volume (50 ml) from methanol extract and it is called methanol concentrates.

The methanol concentrate may contain tar, moisture, flavonoids, steroids etc. Hence, it is washed by petroleum ether and with few drops of dilute H_2SO_4. Oil like residue removed and the methanol concentrate filter by multi layered No.1 waterman paper. Petroleum ether and methanol were evaporates and the brownish past like filtrate obtained and it was again dissolves in to the pure ethanol Then it is called methanol filtrate.

Preliminary Phytochemical screening:

The main objective of the preliminary phytochemical screening is to investigate the plant extract in terms its active constituents. It involves the partial isolation of active constituents and to identify them qualitatively. In this screening various types of identification tests for a variety of chemical classes have been performed according to Ayurveda pharmacopoeia.

Analytical TLC for identification:

Analytical TLC was carried out on preparative TLC plates (5 × 5 cm with 0.2mm thickness, silica gel GF_{254}, Merck, Darmstadt, Germany) cut from the commercially available sheets. An aliquot of standard solution of Quercetin and a sample solution of crude extract was spotted onto the silica gel plate and allowed to dry for a few minutes. Afterwards, the chromatography plate was developed with Chloroform: Acetone: Formic acid (76:16.5:8.5v/v) as mobile phase in a previously saturated glass chamber with eluting solvents for some time at room temperature. The developed plate was dried under normal air and the spots were visualized by spraying with a solution of 0.5%w/v ferric chloride and dried under oven. The Rf (retention factor) values of isolated compounds and standard were calculated and compared.

Recommended procedure for determination of b lapachone in methanolic and acetonic extract by uv-spectrophotometry

Determination of λ max:

The UV-visible spectra of various diluted solutions of β-lapachone in mobile phase were recorded using UV-visible spectrophotometer. The peak of maximum absorbance was observed at 207 nm. This wavelength was used for detection of β-lapachone.

Method validation

Validation is a process of establishing documented evidence which provides a high degree of assurance that a specific activity in consistently desired result, or a product meeting is predetermined specifications and quality characteristic. The method was validated for different parameters like linearity, accuracy, precision, specificity, and ruggedness, limit of detection and limit of quantification.

Linearity:

For each solvent various aliquots were prepared from the stock solution ranging from 25 – 125µg/ml. The sample analysed with the help of UV-VISIBLE spectrophotometer using a respective solvent as a blank. The linearity of the above mentioned sample can be observed in regression equation and correlation coefficient were determined.

Accuracy:

The accuracy of the method was determined by preparing solutions of different concentrations that is 80%, 100%, 120% in which the amount of drug was kept constant i.e., 50µg and amount of pure drug was varied., that is 40µg, 50µg, 60µg for 80%, 100%, 120% respectively. The solutions were prepared in triplicate and the accuracy was indicated by % recovery.

Precision:

Intraday and Interday precision study of β-Lapachone was carried out by estimating responding responses 3 times on the same data and on different days for the concentration of 10 µg. The percentage relative Standard deviation (%RSD) was calculated which is within the acceptable criteria of not more than 2.0.The results for Interday precision.

Limit of detection:

The detection limit is determined by the analysis of sample with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

LOD=3.3(SD/S)

Where SD= the standard deviation of the response

S= the slope of the calibration curve

Limit of quantification:

The quantification limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precise.

LOQ=10 (SD/S)

Where SD= the standard deviation of the response

S= the slope of the calibration curve

Assay:

From the Stock solution of Standard and Sample, 1ml was pipette out into 10 ml volumetric flask individually. It was dissolved using 85% ethanol and made up to the mark with the same to get a solution of 10µg/ml of each. Measure the Standard and sample absorbance's and calculates the % assay.

Amount=concentration*Dilution factor

Validation of the developed method:

The effect of wide range of other constituents and other additives usually present in the extract was investigated to know the specificity of the method. It shows no interference from other compounds. For linearity**,** Aliquots of primary working standard solutions containing b-lapachone were diluted such a way that the final concentrations of b-lapachone are in the range of 25-100 µg/ml. A calibration curve was plotted between concentration and peak area response and statistical analysis of the calibration curve was performed. Method of least square analysis was carried out for getting the slope, intercept and correlation coefficient, regression data values. Precision was determined by intra-day and inter-day study. Precision of the method was evaluated by carrying out the assay and analyzing corresponding responses 6 times on the same day and on different days for the sample solution. The percent relative standard deviation (% RSD) was calculated. Accuracy studies were performed for b-lapachone at three different levels (80%, 100% and 120%) and the mixtures were analyzed in triplicate by the proposed method. Known amount of standard b-lapachone at 80%, 100% and 120% of sample (which was previously analyzed) was added and it was preanalysed by the proposed method. And the percentage recovery was evaluated. Limit of Detection and Limit of Quantitation were calculated using following formula LOD= 3.3(σ)/S and LOQ= 10 (σ)/S, Where (σ) = standard deviation of response (peak area) and S= slope of the calibration curve.

RESULTS AND DISCUSSION

Complete extraction of β-Lapachone was achieved by successive solvent extraction with methanol. Preliminary phytochemical study reveals that the extract may contain phenolic compounds which may be flavonoid, tannins, coumarone glycosides, alkaloids, cardiac glycosides, steroids, inulin, and volatile oils in nature. Several mobile phase combinations were tried and Toluene: Ethylacetate (70:30v/v) was found optimum for separation of β-Lapachone from methanolic extract of Tecomaria Capensis. The R_F values of standard and sample compound matches each other and the R_f value was found as 0.36. TLC profile of compound was represented. Isolation of β-Lapachone from the extract was achieved by preparative thin layer chromatography using the same chromatographic conditions followed for identification of active constituent

CONCLUSION

Identification and UV spectrometry estimation of β-Lapachone was achieved successfully which will be helpful for the standardization of herbal formulations containing this active constituent. The proposed UV method is linear, accurate and precise and can be adopted for the determination of concentration of B Lapachone in various samples from various herbs and formulations with shorter run time and good efficiency.

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FIGURES AND TABLES:

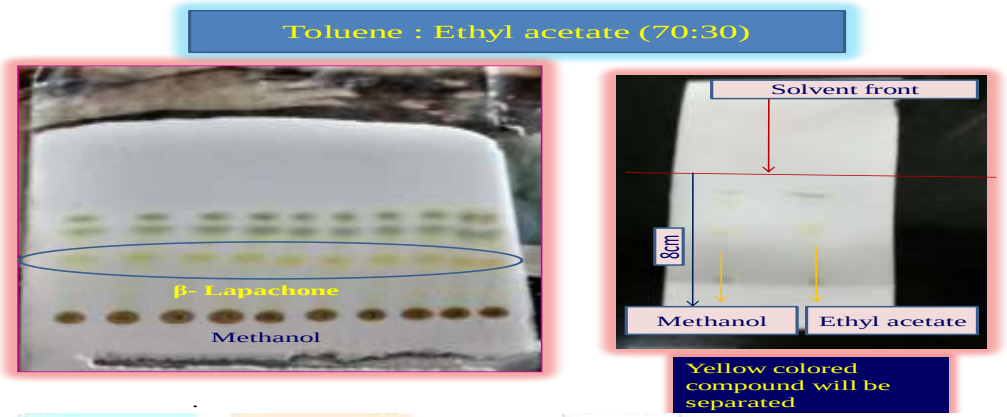

Figure- 1 TLC shows Separation of Compounds in7:3 Ratio of Toluene, Ethylacetate.

Figure-2

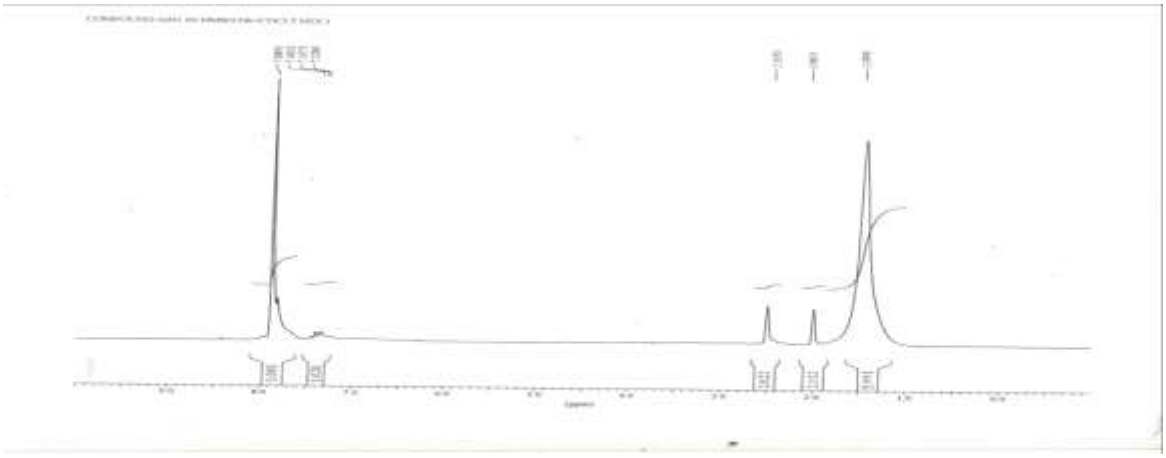

Figure-3

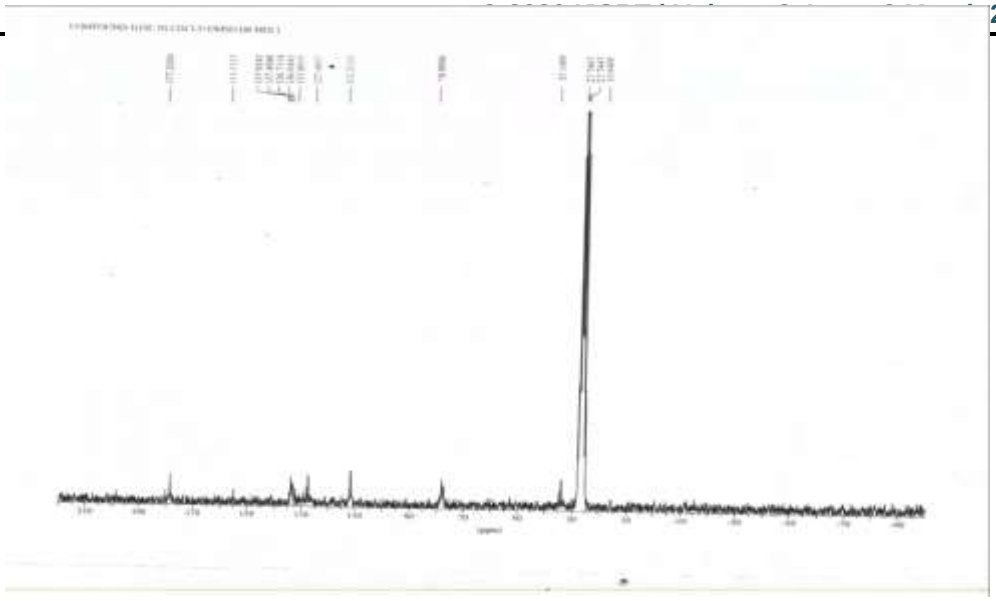

Figure:4

Figure:5 Absorption curves of leaf extract

Table No.1 Proximate analysis

NAME OF ASH VALUE	VALUE (in gm)
Total Ash Value	0.93
Acid Insoluble Ash Value (dil.Hcl)	0.01
Sulphated Ash Value (H_2SO_4)	0.06
Water Soluble Ash Value (H_2O)	0.05

Table No.2 Different Extractive Values

NAME OF EXTRACTIVE VALUE	VALUE (in gm)
Alcohol Soluble Extraction	0.25 gm
Water Soluble Extraction	0.36 gm
Methanol Soluble Extraction	0.04 gm

Table No. 3 Percentage Yields of Different Extracts of Tecomaria capensis Leaves.

S.NO.	SOLVENT	NATURE OF EXTRACT	COLOUR	% YIELD
1	Pet. Ether	Semisolid	Dark Yellow	0.35
2	n-Hexane	Semisolid	Dark Yellow	0.13
3	Chloroform	Semisolid	Dark Green	0.20
4	Ethylacetate	Semisolid	Dark Green	0.70
5	Methanol	Semisolid	Dark Green	0.52
6	Aqueous	Semisolid	Dark brown	0.25

Table No. 4 Preliminary phytochemical screening

Phyto constituents	Pet. Ether	n-hexane	Chloroform	Ethylacetate	Ethanol	Water
Alkaloids	--	--	--	--	++	--
Flavinoids	--	--	++	++	++	++
Cardiac Glycosides	++	++	++	++	++	--
Saponin Glycosides	++	++	++	++	--	--
Coumarin Glycosides	--	--	--	++	++	--
Tannins	--	--	--	--	++	--
Steroids and Terpinoids	--	++	++	++	++	--

Carbohydrates	--	--	--	++	--	--
Protein	--	--	--	++	++	--
Inulin	--	--	--	--	++	++
Volatile oil	++	++	++	++	++	--
Waxes	--	--	--	--	--	--
Mucilage	--	--	--	--	++	++

Table No. 5 Linearity result of β-Lapachone

S. No.	Concentration of standard (µg/ml)	Absorbance at 207.1nm
1	0	0
2	0.2	0.0393
3	0.4	0.0703
4	0.6	0.1053
5	0.8	0.1362
6	1	0.1737

Table No. 6 Absorbance of leaf extract

S.NO	Name of the extract	Absorbance	Absorption maxima(nm)
1	Methanolic extract of β-Lapachone	2.831	144

Table No. 7 Result of validation parameters

S.NO	Parameters	standard β-Lapachone	Methanol extract
1	Absorption maxima(nm)	207	144
2	Slope	0.170	0.007
3	Intercept	0.002	0.007
4	Correlation coefficient(R^2)	0.999	0.998
5	Range	0.2-1.0ug/ml	0.2-1.0ug/ml
6	Regression equation	Y=0.170 X-0.002	Y=0.0076 X-0.0072
7	Limit of detection	5.327 ug/ml	0.417ug/ml
8	Limit of quantitation	10.27ug/ml	1.237ug/ml
9	Result of Assay (%)	99.8	98.7

Table No. 8 Result of precision

S.No	Inter day	Intraday		
		Day1	Day 2	Day 3
1	0.0393	0.0394	0.0393	0.0393
2	0.0394	0.0394	0.0393	0.0392
3	0.0393	0.0393	0.0392	0.0393

Table No. 9 Results of Precision

Drug	Interday precision %Amount found±S.D	%RSD	Intraday precision %Amount found±S.D
β-Lapachone	98.46 ±0.20	0.242	97.52±0.312
β-Lapachone	98.83±0.27	0.512	98.23±0.396

Table No. 10 Result of Accuracy

Percentage level	%Recovery± S.D	%RSD
80%	97.3±0.008	0.303
100%	99.5±0.007	0.412
120%	99.5±0.0081	0.687

Table No: 11 Result of Ruggedness

Std drug	Analyst I	Analyst II
0.2	0.0392	0.0393
0.4	0.0702	0.0701
0.6	0.1052	0.1051
0.8	0.1361	0.1360
1	0.1736	0.1735

Table No. 12 Result of Ruggedness

Name of the extract	Analyst1	Analyst II
Methanolic extract of β-Lapachone	0.004	0.004

Table No. 13 Result of extract Assay

Name of the extract	Amount found (g/ml)
Methanol	0.040

Table No: 14 Result of assay

Extract of β-Lapachone	%Assay
Methanol	98.31

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