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Research Article

QUALITY EVALUATION OF NYCTANTHES ARBOR-TRISTIS EXTRACTS BY UV-VIS SPECTROSCOPY AND HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY TECHNIQUES

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ABSTRACT:

Nyctanthes Arbor-tristis is one of the most useful medicinal plants in India. Each part of the plant has some important medicinal value and is thus commercially exploitable. The popular medicinal use of this plant is anti-inflammatory, anti-pyretic, anti-viral, anti-bacterial, anti-allergic and diabetes control and it is used as several diseases. The present research is to isolate plant pigments by different extraction techniques and to focus on the identification and determination of the purity, quality, quantity by using UV-Visible spectroscopy and High performance thin-layer chromatography technique. From the results obtained by the above analytical techniques it is observed that Soxhlet extraction technique showed better results in HPTLC when compared to masceration technique.

Keywords: Nyctanthes arbor-tristis, anti-inflammatory, anti-pyretic, UV-visible spectroscopy, HPTLC.

INTRODUCTION

UV-VIS Spectroscopy

Nyctanthes arbor-tristis Linn is known to be one of the extremely useful medicinal plants in South Asia. The popular medicinal use of this plant is anti-helminthic and anti-pyretic besides it is used as laxative, rheumatism, skin alignment and as a sedative. Vitally, it is native plant present in the home gardens to pass on its medicinal uses to on coming generations. It was observed that the leaves of the plant are used in treating fever. The present work is an attempt to make isolation of the phytochemical constituent and mechanism of pharmacological actions with focus on pyretic conditions. The present study includes comprehensive information on the chemical constituents, biological activities of important compounds, pharmacological actions, medical applications and micro propagation of night jasmine and emphasizes the need in Ayurveda Nyctanthes arbor-tristis Linn.is termed as mythological plant with enormous medicinal value. The present study was performed for establishing the quality standards of the leaves as per World Health Organization (WHO) guidelines to confirm its purity and authentication. Leaf juice is protected laxative for new born children and is utilized in treatment of steady fevers mix with various species. This article gives the suitable paediatric emulsion for antipyretic activity. The chemical constituents (terpenoids) are responsible for potent antipyretic activity. Extract where concentrated analysed by UV (ultra violet spectroscopy) for checking ability of the chemical compounds to absorb UV (ultra violet spectroscopy) light and it shows absorption features in the visible region. This technique is to investigate and quantify leaf extract and useful medicinal compounds. This study highlights crucial role of terpenoids in the formulation. From all observations it shows this paediatric emulsion has the antipyretic activity.[1,8]

HPTLC

The herbal medicines have reached extensive acceptability as therapeutic agents for various clinical diseases due to global demand. Therefore, standardization is the essential and initial step to drug development. It is for the establishment of consistent biological activity, a consistent chemical profile and biomarker identification. It improves the safety and efficacy of herbal medicine to provide the best herbal medicine to society and increase popularity rather than non-standardized extracts. In addition, it is essential to practice or maintain a quality assurance program for the production and manufacturing of herbal medicine that includes the basis of organoleptic characters and photomicrographs, physicochemical, proximate analysis phytochemical evaluation

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and quality control analysis and order to assess the quality of drugs, based on the concentration of their active principles. WHO has provided specific guidelines for the assessment of the safety, efficacy and quality of herbal drugs as a prerequisite for global harmonization and of utmost importance. In the present study, the herbal extracts were cleaned, dried in the shade and powdered by passing through the sieve as per the method described in the standard protocol. An overview covering various techniques employed in the extraction and characterization of Nyctanthes arbortristis, Hippophae salicifolia, Ocimum tenuiflorum and Reinwardtia indica, standardization is reported in this study. The obtained data would be very significant for future clinical aspects, as the bioactive molecules present in the extracts may exhibit synergistic effect with other bioactive compound and show a better therapeutic value. Thus, this study provides standardized and therapeutically potential data of active polyherbal formulations for the different ailments. [2,3,4]

Materials and methods:

1. Collection of Plant Samples [leaves]:

Fresh leaves were collected randomly from surrounding regions. The plants were identified and studied according to their families. Collected leaves were washed under the tap water, shade dried and then homogenized to fine powder and stored in an air tight container.

2. Preparation of leaves extract:

- Fresh leaves were collected from Nyctanthes Arbor-tristis and gently washed to remove dust particles.
- Later the leaves were shade dried at room temperature for 20 days and grinded to make a fine powder.
- After making a fine powder go for the extraction process.

a.Cold maceration process

b.Soxhlet extraction process

a.Cold maceration process

- About 1kg of dried powdered leaves of the plant was extracted by cold maceration using ethanol as solvent for 7days, with intermittent agitation.
- The extract was collected and filtered by using filter paper.
- Then the filtrate was distilled (steam distillation) to remove the excess solvent (ethanol).
- The procedure was repeated until the plant material is completely extracted.
- The concentrated extract is collected and Phyto-chemical screening was performed to identify the chemical constituents.

b.Soxhlet extraction process

Soxhlet extraction is a chemical technique that uses a solvent to continuously extract a compound from a solid material. It's a well-established method for extracting solid samples with limited solubility in a solvent, even when there are insoluble impurities.

Steps

1. The finally grounded leaves of 10g were placed in a thimble with paper bag or placing cotton in the thimble below and above the grounded leaves.
2. Connect Soxhlet with the condenser to condense the extract evolved during the Soxhlet extract evaporation.
3. Now connect the Soxhlet with RBF and place the Soxhlet apparatus containing RBF in a water bath.
4. Fill the thimble with 100ml of ethanol as a solvent.
5. Maintaining the temperature below 25^0C to avoid the degradation of the chemical constituents which are heat stable.
6. The extract was collected after the series of 7 cycles.
7. Collect the extract from the RBF and run for the analysis of chemical constituents.

The collected extraction product was run for the analysis UV-VIS Spectroscopy, TLC (Thin layer chromatography) and HPTLC (High performance thin layer chromatography) as shown in table 2.

UV-VIS SPECTROSCOPY METHOD

In UV-VIS spectroscopy, the transition of electrons at various levels by absorption of radiation from ultraviolet to visible region is plotted in a graph. This line graph of various absorptivity's on specific levels of radiation is because of the absorption capacities of compounds at certain levels. These levels are called regions of absorption, and the compounds are termed chromophores. chromophores are present in almost every compound. This can be deduced by the fact that almost all compounds and especially organic compounds can be identified and quantified by the UV-VIS spectroscopy.[5]

Instrument: UV-VIS SPECTROSCOPY (LAB INDIA) with UV analyst Software.

Procedure

Preparation of samples (Soxhlet extract)

- Collected extract was diluted into different concentrations.
- UV-visible spectra of fractions of leaves extract. 0.2µg/ml 0.4µg/mL, 0.6 µg/mL, 0.8 µg/mL, 1µg/mL of 20%, 40%, 60%, 80%, 100% of samples were respectively used for spectral studies.

- Distilled water is used as blank in the spectral study of Nyctanthes arbor-tristis.

Selection of wavelength for analysis: By appropriate dilutions of sample solution to 1µg/ml these diluted solutions were scanned in the range of 200-800nm using UV-VIS Spectrophotometer. Shown in Fig 1s

Observation

The sample solution showed λ max at 252 nm with distilled water as a blank as shown in table 1

UV spectra of collected extract:

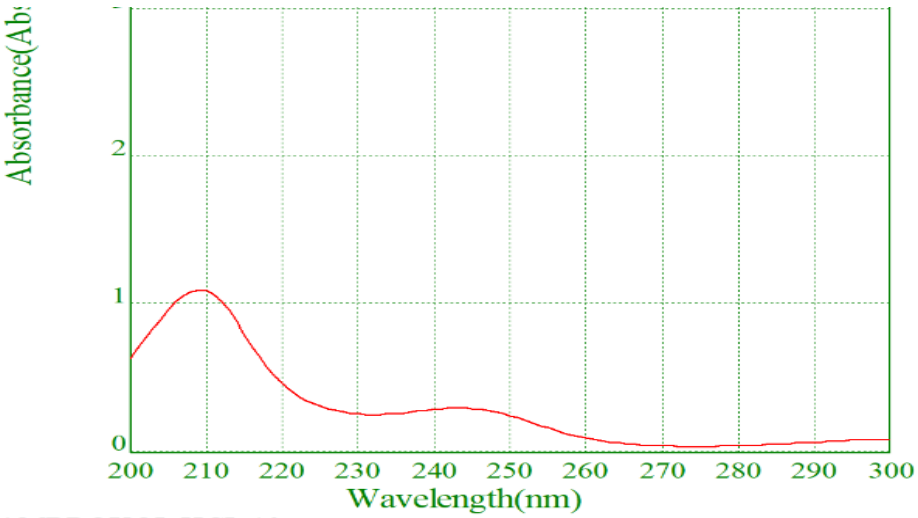

Figure.1: UV Spectra of extract Nyctanthes arbor-tristis

Table 1: Linearity data by UV Spectroscopy

S.NO	CONCENTRATION (µg/ml)	ABSORBANCE
1	2	0.3568
2	4	0.6722
3	6	0.9462
4	8	1.2869
5	10	1.4701

TLC METHOD

The separation of components in sample were clearly observed in the TLC analysis with mobile phase ratio - Ethyl acetate: Methanol: Distilled Water (30:5:5)

So we further performed HPTLC for quantitative analysis of the separated compounds obtained since it is not possible in TLC.[6]

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)

High performance thin layer chromatography (HPTLC) is sophisticated instrumental technique based on the full capabilities of thin layer chromatography. HPTLC have a similar approach an employe the same physical principles of TLC i.e the principle of separation is a adsorption. The main difference between them is in the characteristics of the separation plate. HPTLC plates are based on optimize silica gel 60 with a significantly smaller particle size than used for classical TLC. This allows a higher packing density and a smoother surface as shown in figure 2, 3, 4, 5, 6. Hence, sample diffusion is reduced, resulting in compact bands or spots. Furthermore, the smaller particle size and thinner layer significantly increased detection sensitivity and analysis speed. The solvent consumption is less in twin through chambers as compared to the flat bottom ones.[7]

Instrumentation

Chromatographic conditions:

Instrument - Camag Linomat 5 HPTLC, with Wincats software.
Spray gas - Inert gas
Sample solvent type - Methanol
Chamber type - Twin trough chamber 20×10cm
Mobile phase - Ethyl acetate: Methanol: Distilled water (30:5:5)
Temperature - 500C
Time - 5mins
Wavelength - 254nm
Lamp - D2 & W

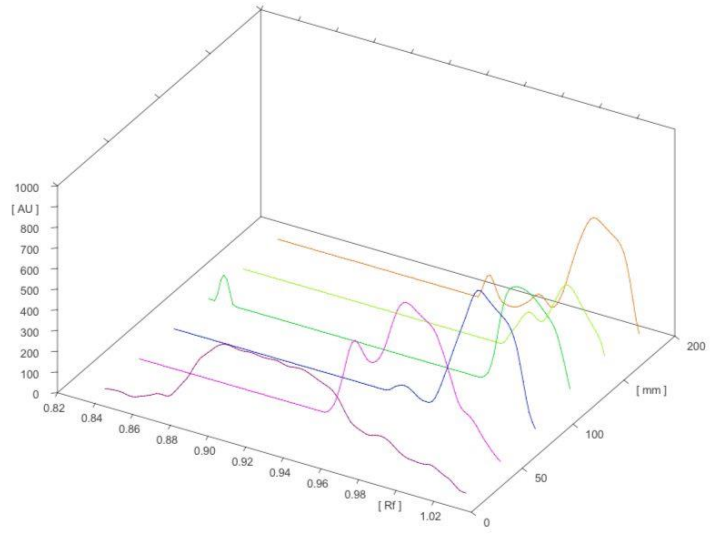

Figure.2: HPTLC – Densitogram of different extraction techniques of Nyctanthes Arbor-tristis.

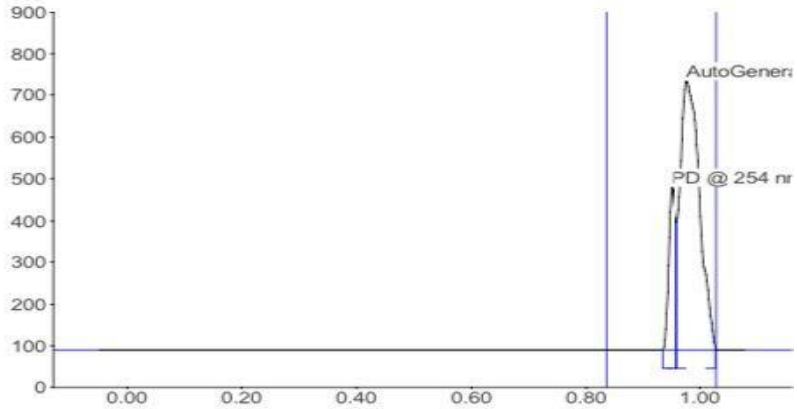

Figure.3: Maceration extract

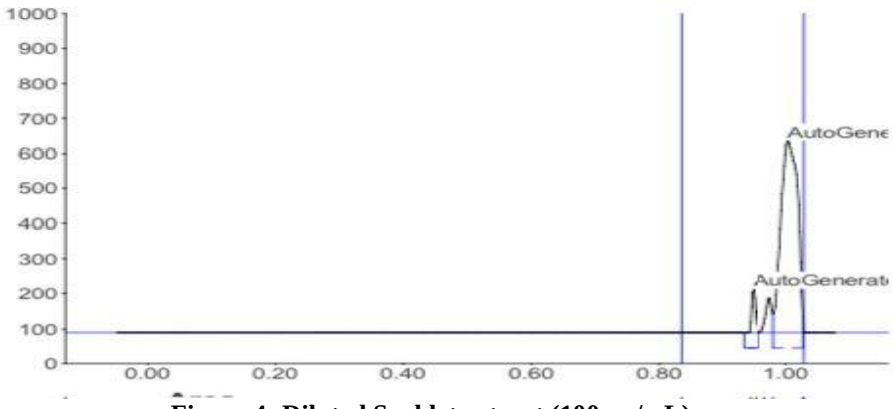

Figure.4: Diluted Soxhlet extract (100 µg/mL)

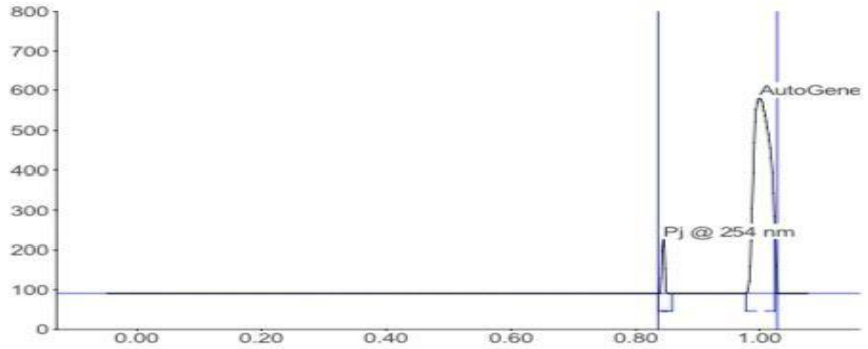

Figure.5: Soxhlet extract (1000 µg/mL)

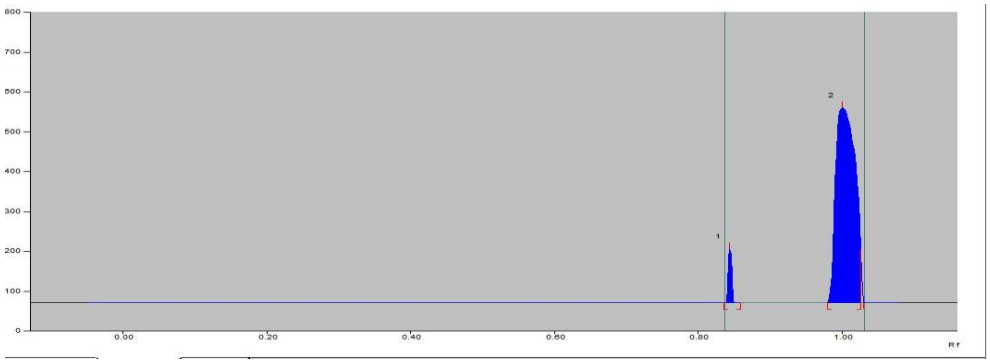

Figure.6: Densitogram of Soxhlet extract

Table 2: Results obtained from HPTLC analysis of extract

Sample	RF Values	Area
Maceration extract	0.96	15787.2
Diluted Soxhlet extract	0.97	10343.9
Soxhlet extract	0.98	9561.1

Conclusion:

By using UV-VIS spectroscopic technique, sample showed maximum absorbance at 254 nm wavelength. It allowed for the identification of key absorption peaks, indicative of specific phytochemical constituents, and helped in the preliminary assessment of extract purity. In the HPTLC analytical technique we have determined the peak areas, quality and quantity of the compound in the Nyctanthes arbor-tristis extract by using Rf values. Further we will determine the specific active compound present in the sample, by comparing with a standard by using this developed HPTLC method.

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