RESEARCH ARTICLE

Formulation and Standardization of Essential Phytochemicals from *Calotropis gigantean* Oil by Conventional Hydro Distillation Method

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ABSTRACT

In Ayurveda, herbal remedies are an essential component of treatment. Correct botanical identification becomes the most important requirement for their appropriate clinical use. Over 80% of people worldwide rely on traditional medicine for their primary medical treatment, according to the WHO. 'Herbal medicines' are safer than expensive synthetic treatments, which is the fundamental reason for the rising interest in plant-derived medications. Various plant varieties are described in ancient classical literature according to their flower colors, sizes, and shapes. A plant (family: *Asclapiadacea)* is shrub has long been used to cure a variety of illnesses, including as hepatic, analgesic, and anti-inflammatory conditions. The pharmacological potential has not yet been thoroughly investigated. The plant's leaf has not been studied for its anti-paralysis properties, according to a critical analysis. The goal of the current study is to screen for phytochemicals and prepare herbal oil for formulation and standardization of essential phytochemical of calotropis gigantean oil by conventional hydro distillation method, which is a somewhat difficult process that calls for extra caution with regard to ingredient proportions, heating methods, and temperature. Additional chromatographic estimation was performed on the *Calotropis gigantean* Oil formulation and it has set the standard as novel formulas for the treatment of paralysis diseases.

Key words: 'Herbal medicines, Herbal oil, *Calotropis gigantean* Oil, HPTLC, Paralysis diseases

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INTRODUCTION

Plants have important role in giving medicinal value. Ever since ancient times plants are familiar to cure illness or strengthen physical health in humans. The evolution of human civilization has been significantly influenced by medicinal plants. For practically all cultural civilizations, medicinal plants have been a major source of medicine. Many modern medications are made from medicinal plants, which are regarded as a rich source of traditional medicines. Therapeutic plants and their qualities have been utilized for thousands of years to treat illnesses, preserve food, provide flavor, and stop epidemics. Even today, plants are noted to have a wide range of treatments to physical complications of the human body. In China 40% of medicine comes from plants and in Asian countries it is 80%. Numerous classic medicines are plant based, a number of the primary effective medicine are primarily originated from plant sources like painkiller taken from bark of willow and painkiller taken from the poppy. A branch of science subsidizing to the study of medicinal uses of plants to facilitate for future medicines is referred to as Ethnobotany.[1-7] Herbal oils, additionally referred to as plant oils, constitute a blended fraction of fats-soluble i.e. lipid soluble compounds obtained from seeds, fruits, flowers, roots, bark, or leaves. The composition and residences of natural oil are decided via way of means of the kind and a part of the herb used, in addition to the manner of production[8-12].

If the lipid soluble content of plant ingredients is less then they will be dissolved, extracted, and separated from the plant with the help of a suitable solvent. The aggregate of lipid soluble natural ingredients extracted and separated from the herb into a suitable solvent is natural oil extract. Fat-soluble nutrients, several carotenoids, flavonoids, polyphenols, phytosterols, and different vitamins had been observed and remoted from natural oil extracts, Because "herbal medicines" are safer than pricey synthetic prescriptions, there is a growing interest in plant-based or natural medications. *Calotropis gigantea*, commonly referred to as "Great Calotropis" or "Rui" in India (Figure 1), was chosen as the plant for this investigation. Alkaloids, glycosides, flavonoids, and terpenoids were among the secondary metabolites found by phytochemical screening. This investigation demonstrated the plant's substantial pharmacological value and emphasized its significance. The leaves are utilized to relieve joint discomfort and paralyzed areas. In addition to treating ringworm and leprosy, milky latex is also used to cure arthritis, cancer, and snake bites.[13-17]

MATERIALS AND METHODS

Collection of plant part

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Leaves of Calotropis gigantea were collected from Durga Tekdi, Nigdi. Leaves were collected randomly. Herbarium was prepared and was authenticated by BSI, Pune.

Preparation of oil

Wash the leaves to remove the contaminants and shade dry the leaves of *Calotropis gigantea*. Grind the dried leaves into powder. The powder should be coarse in texture. Store the powder in a clean zipped plastic bag. Tila oil (4 parts) was prepared by heating it on a heating mantle until the foam vanished in a round-bottom flask (Table 1). Heating was continued till the water completely evaporates.[18] Temperature during heating the mixture must be stable. Then water (16 parts) was added. The ratio of Tila oil to that of water was 1: 4. The powdered drug of the leaves was

Figure 1: *Calotropis gigantean*

Figure 2: Heating of leaf extract

Figure 3: Filtered oil extract

Table 1: Ingredients used in oil extraction

S. No	Material used	Quantity taken
1	Leaf powder	18.7 g
2	Teel oil	74.8 ml
3	Water	300 ml
Total oil extract		44.5 ml

added to the mixture of oil and water. The mixture was then heated and the heating was continued till the mixture attains "perfect wick shape" when rolled between thumb and index finger. Or upon heating the prepared oil should not produce any crackling sound on fire. The mixture was pressed by a dry cloth to extract all the oil from the mixture. Filter the oil extract. The oil was collected and stored in a clean and dry container. Figure 2 shows the heating of leaf extract and Figure 3 shows the Filtered oil extract

Evaluation and standardization

Specific gravity

Since oils are lighter than water, they should have a specific gravity of less than 1. The specific gravity container should be rinsed with distilled water, dried in the oven for fifteen minutes, allowed to cool, sealed with a cap, and weighed (a). Weigh the sample again after capping it and transferring it to the same specific gravity bottle (b). Calculate the sample weight per milliliter by deducting the weight[19] (b-a) results are shown in table 2

Saponification Number

Two grams of oil were carefully weighed and then added to a 250-milliliter iodine flask. After adding 25 milliliters of 0.5M alcoholic potassium hydroxide, the liquid was cooked on a reflux water bath for 30 minutes. The phenolphthalein was added as an indicator and then titrated against 0.5M HCl ('a' ml).[20] In a similar manner, blank ('b' ml) was carried out without sample results are shown in table no.2

pH

It is determined by digital pH meter. Results are shown in table no.2

Acid value

ten milliliters of oil have been included along with 25 milliliters of ether and ethanol. A 0.1M potassium hydroxide solution was used to titrate the indicator phenolphthalein results are shown in table no.2

Color and odor determination

the oil has a pungent smell and a vivid green tint results are shown table 2

Clarity test

to determine if the herbal oil is clear or not, a clarity test was conducted results are shown in table no.2

Instrumentation

UV-Visible spectrophotometer (Shimadzu 2450); Program Linomat 5, HPTLC System CAMAG (Muttenz, Switzerland), and Camag TLC Scanner 3 were utilized.

Reagents and chemicals

Analytical-grade chemicals and reagents were all utilized (MERCK Chem. Ltd., Mumbai).

Preparation of sample

100 milliliter volumetric flasks were filled with ten milligrams of carefully weighed oil. To reach a final strength of 100 µg/mL, it was dissolved in methanol and the volume was adjusted using the same solvent.

Table 2: Evaluation parameters of herbal oil

S.no	Parameters	Values
1.	Specific gravity	0.85
2.	Saponification value	168.3
3.	Acid value	4.2
4.	pH	5.9
5.	viscosity determination	5 Rpm – 2580 cP 10 Rpm – 1290 cP 20 Rpm – 645 cP 30 Rpm – 430 cP 40 Rpm – 322.5cP 50 Rpm – 258 cP 60 Rpm – 215 cP 70 Rpm – 184.3 cP 80 Rpm – 161.2 cP 90 Rpm – 143.3 cP 100 Rpm – 129 cP 150 Rpm – 86 cP 160 Rpm – 80.6 cP 200 Rpm – 64.5 cP
6.	Determination of color	Light green
7.	Determination of odor	Pungent
8.	Clarity	clear

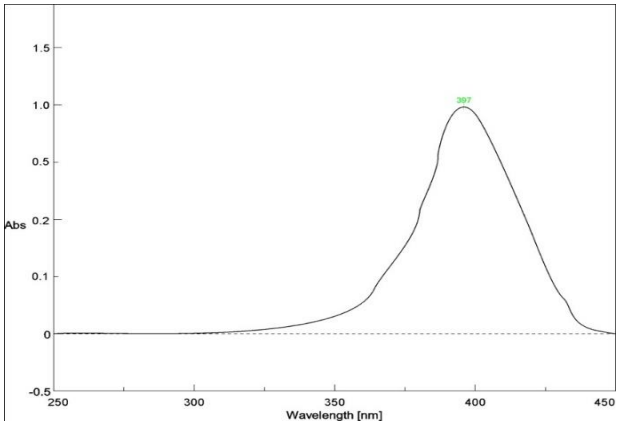

Figure 4: UV peak of oil extract

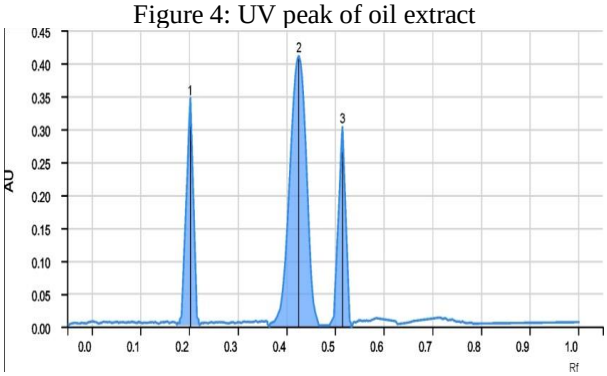

Figure 5: R_f Peak of oil extract

Stationary Phase

For identification and determination, stationary phase 60 F254 S TLC plates (20 cm × 10 cm, layer thickness 0.2 mm, E-Merck, Darmstadt, Germany) with an aluminum backing.

Optimization of mobile phase

As the mobile phase, various ratios of n-hexane, toluene, methanol, ethyl acetate, and glacial acetic acid were explored; however, in the majority of the attempts, tailing of spots and less persistent spots were seen.

Chromatographic conditions

Chromatography was conducted using 10 cm × 10 cm aluminum-backed TLC plates that had been covered with 200 µm layers of silica gel 60F254 S. The plates were prewashed with methanol and activated at 100 to 110 °C for 10 minutes before to chromatography. Using a hundred µL sample syringe and a Linomat-5 sample applicator, the plates were covered with the samples in broad bands of 6 mm.

RESULTS AND DISSCUSSION

Determination of wavelength by UV

The right amount of volume A 10 ml volumetric flask was filled with 0.2 ml of the standard stock solution of calotropis oil that had been diluted with methanol to a concentration of 2µg/ml. The final solution was scanned in the UV range of 200–400 nm. Calotropis oil's absorbance peaked in the spectrum at 397 nm (Figure 4).

HPTLC

Toluene: n-hexane: glacial acetic acid (8:1.5:0.5) was tried to solve the issues, and the results show a crisp, symmetrical peak with an Rf value of 0.4202 and good resolution. Using toluene, n-hexane, and glacial acetic acid (8:1.5:0.5) as the mobile phase, the plate was developed to a distance of 8 cm in a Camag twin-trough glass chamber that had been saturated with mobile phase vapors for 10 minutes at room temperature. The Camag TLC Scanner 4 with visioncats software was used to do densitometric scanning at 397 nm. Results are shown in Figure 5 displays a typical Calotropin chromatogram.

CONCLUSION

The study aimed to investigate the potential of *calotropis gigantea* leaves extract in terms of its phytochemical compounds as an anti- paralysis, According to the results obtained from specific gravity, saponification value, acid value, pH, viscosity determination, color identification, odor and clarity evaluation, UV and HPTLC charaterisation of *calotropis gigantea* leaves oil formulation, shown the good and satisfactory and it has been set as standard parameters for oil formulation of *calotropis gigantea* leaves. As per ethno botanical survey this plant have ability to cure paralysis. So, the herbal oil of *calotropis gigantea* was prepared and standardized its phytochemicals compared with standard markers. Herbal oil formulation was standardized by UV and HPTLC method.

REFERENCES

1. Doughari JH, Phytochemicals: Extraction methods, basic structures and mode of action as potential chemotherapeutic agents. Croatia: Intech Open Access Publisher. 2018;16(4)1-33
2. Mikawlrawng K, Rani R, Kumar S, Bhardwaj AR, Prakash G. Anti-paralytic medicinal plants–Review. Journal of Traditional and Complementary

Medicine, 2018; 8(1): 4-10. doi: 10.1016/j.jtcme.2017.02.001.
3. Timilsina H, Modi B, Basnyat R. Phytochemical, antimicrobial and ethnobotanicalstudy of *Calotropis gigantean*. Journal of Health and Allied Sciences; 2020; 10(2): 23-27. DOI:10.37107/jhas.136
4. Al Sulaibi MA, Thiemann C, Thiemann T. Chemical constituents and uses of Calotropisprocera and Calotropisgigantea–a review (Part I–the plants as material and energy resources). Open Chemistry Journal, 2022; 7(1): 154-158. DOI: 10.2174/1874842202007010001
5. Chopade VV, Gawade VS, Puri MV, Hol KC, Chaudhari RG. Synthesis of thiophene hydrazone derivatives by Japp Klinegmann reaction method for anticancer activity against MCF-7 cell line. International Journal of Pharmaceutical Quality Assurance. 2024;15(3):1661-1665. DOI: 10.25258/ijpqa.15.3.86
6. Abood MA, Kadhim EJ. Phytochemical investigation of some active components in Iraqi Conyza canadensis (Syn. Erigeron canadensis). International Journal of Drug Delivery and Technology. 2021;11:669-675.
7. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Latha LY. Extraction, isolation and characterization of bioactive compounds from plants' extracts. African journal of traditional, complementary and alternative medicines. 2011;8(1).139-146
8. Rai P. Concept of taila Kalpana in ayurvedic pharmaceutics–A critical review. Unique Journal Ayuvedic Herbal Medicine. 2015;3:79-81.
9. Panda P, Das B, Sahu DS, Meher SK, Bhuyna GC, Das BK, Rao MM. TailaKalpana (Medicated Oil) in Ayurveda. Research Journal of Pharmacology and Pharmacodynamics, 2016;8(1): 39-41.
10. Chopade JV, Hujare DP. Biocompatibility and Haemocompatibility Assessments of RBC with Polymeric Coated Cardiac Stent. International Journal of Pharmaceutical Quality Assurance. 2024;15(3):1650-1653.
11. Sabri LA, Hussein AA. Oral Liquid Self-nanoemulsion of Nebivolol: Formulation and In-Vitro Characterization for Dissolution Rate Enhancement. International Journal of Drug Delivery and Technology. 2021;11(3):1083-1091.
12. Al-Tamimi DJ. Hussein AA. Preparation and In-vitro Characterization of Tacrolimus as a Solid Selfmicroemulsion. International Journal of Drug Delivery and Technology. 2021; 11(1):70-78.
13. Noor HK, Abass SM. Development of a visible spectrophotometric analysis for hydrochlorothiazide in pure and pharmaceutical dosage forms. International Journal of Drug Delivery Technology. 2021; 11(2):496-500.
14. Banpure SG, Chopade VV. Docking study of selected Phytoconstituents with Acetylcholine Esterase and BChE for its Anti-alzheimers Activity. International Journal of Drug Delivery Technology. 2022;12(3):1413-1417.
15. Jain PS, Kale NK, Surana SJ. Stability-Indicating HPTLC Method for Estimation of Guaifenesin in Bulk and in Pharmaceutical Formulation. Journal of Chromatography and Separation Technology. 2015;6(273):26-33
16. Chopade JV, Hujare D. Characterization and In-vitro Study of Polyethylene Glycol as Coating Material used as Drug Carriers on Coronary Stent for Treatment of Cardiac Diseases. International Journal of Drug Delivery Technology. 2024;14(2):955-960.
17. Jain PS, Kale NK, Surana SJ. Quantitative estimation of Rosuvastatin in bulk and tablet dosage form by using area under curve method. Journal of Pharmaceutical and Bioanalytical Science. 2013;4:128-33.
18. Banpure SG, Chopade VV, Chaudhari PD. Anti-Alzhimer Activity of Bay Leaves in Scopolamineinduced Rat Model. International Journal of Drug Delivery Technology. 2023;13(1):17-22.
19. Kamruzzaman M, Sazidur R, Islam MS, Parvin S, Nahar K, Uddin MMN, Ganguly A, Shahriar M. Phytochemical screenings and antithrombolytic activities of two common medicinal plants of Bangladesh. International Journal of Drug Discovery and Technology. 2013;4(1):1-4.
20. Sheetal C, Chopade VV, Vasudha B. Exploring Mixed Hydrotropy Concept in HPTLC Densitometric Estimation of Telmisartan. International Journal of Pharmaceutical Quality Assurance. 2024;15(3):1197-1200.