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Isolation and Characterization of a Novel Flavonoid from *Euphorbia neriifolia*

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Abstract

Background: Many of the vivid colours found in fruits, vegetables, flowers, and even tea are caused by interesting natural substances called flavonoids. Because they appear to provide mild, protective effects that assist our systems in daily ways, these plant-made compounds have become a hot issue in research and health. Flavonoids shield plants from oxidative stress, illnesses, UV rays, and herbivores. E. neriifolia, or Euphorbia neriifolia. There are several ethnomedical applications for E. neriifolia. In addition to treating whooping cough, gonorrhea, leprosy, asthma, dyspepsia, jaundice, enlarged spleen, tumors, bladder stones, gastrointestinal issues, and leucoderma, the latex of E. neriifolia is used as a laxative, purgative, rubefacient, carminative, and expectorant. Brittle, hot, carminative, and useful for treating bronchial infections, tumors, aches, inflammations, and stomach swellings, leaves also improve appetite. The goal of the current study was to identify and isolate the unique flavonoid found in Euphorbia neriifolia leaves. ***Methodology:*** Extracts showing presence of flavonoids will be subjected to column chromatography for further separation of individual compounds. Further characterization of isolated compound was carried out by chromatographic and spectral analysis. ***Result:*** Based on current finding the compound C1 was identified as quercetin, a flavanol compound containing phenolic hydroxyl group and conjugated aromatic rings. ***Conclusion:*** The present study successfully isolated and characterized flavonoid compounds from Euphorbia neriifolia using chromatographic and spectral techniques.

Keywords: Euphorbia neriifolia, TLC, HPTLC & Spectral analysis.

INTRODUCTION

Flavonoids are organic chemicals that are widely found in a variety of natural sources, including fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine. They are distinguished by a variety of phenolic structures. There are efforts underway to extract the flavonoids from these natural chemicals because of their well-known health benefits. Compared to other areas of dietary elements, the quantity of studies on flavonoids has increased exponentially throughout recent years. This rise seems to be connected to the current surge in interest in nutraceuticals, supplements, and healthful diets. Large-scale epidemiological research on the intake of fruits and vegetables, especially flavonoids, and the incidence of cancer, stroke, and coronary heart disease were likely another factor. The flavonoid subgroup that has garnered the most attention is the widely dispersed flavonols, followed by flavanols and, more recently, anthocyanidins and other related polyphenols like resveratrol [1]. They serve antioxidant, anti-inflammatory, antimicrobial, anti-obesity, neuroprotective, cardioprotective and anticancer activities [2]. The 15-carbon skeleton of all flavonoids is made up of two benzene rings (designated A and B) joined by a three-carbon heterocyclic C ring [3]. E. neriifolia is a multipurpose plant with a number of ethnomedical applications. In addition to treating whooping cough, gonorrhea, leprosy, asthma, dyspepsia, jaundice, enlarged spleen, tumors, bladder stones, gastrointestinal issues, and leucoderma, the latex of E. neriifolia is used as a laxative, purgative, rubefacient, carminative, and expectorant. In addition to boosting hunger and treating tumors, aches, inflammations, stomach swellings, and bronchial infections, leaves are brittle, hot, and carminative [4].

E. neriifolia

The current study's objective is to use spectrum analytical methods and chromatographic separation to separate and describe flavonoid chemicals from *Euphorbia neriifolia*.

MATERIAL AND METHODS

Collection and Authentication of Plant Material

The acquisition and verification of plant material are essential initial stages in guaranteeing the quality and repeatability of pharmacognostic and phytochemical research. In this study, fresh leaves of Euphorbia neriifolia were obtained from local medicinal plant nurseries and confirmed cultivation sites. A certified botanist, Dr. Smruti Sohani, Professor at SAGE University in Indore, Madhya Pradesh, inspected the obtained specimens in detail for their morphological traits and confirmed their authenticity. Voucher specimens [PA-ENL-012] was subsequently stored for future reference.

Preparation of Extracts

The powdered plant material will be subjected to extraction using solvents of increasing polarity such as petroleum ether, chloroform, ethyl acetate and hydroalcoholic solvent. Extraction may be carried out using Soxhlet apparatus or maceration method depending on suitability. The obtained extracts will be concentrated using rotary evaporator to remove solvent. The percentage yield of each extract will be calculated and recorded.

Preliminary Phytochemical Screening

The prepared extracts will be screened for presence of important secondary metabolites such as flavonoids, alkaloids, tannins, glycosides, saponins and phenolic compounds using standard qualitative tests. Specific tests such as Shinoda test and alkaline reagent test will be performed to confirm presence of flavonoids in plant extracts.

Thin Layer Chromatography Analysis

Thin layer chromatography (TLC) will be carried out for separation of flavonoid components present in extracts. Suitable solvent systems will be selected to obtain good separation of compounds. Developed TLC plates will be observed under UV light at 254 nm and 366 nm to identify flavonoid spots. Retention factor (Rf) values will be calculated for identification of fractions containing flavonoids.

Column Chromatographic Separation

Extracts showing presence of flavonoids will be subjected to column chromatography for further separation of individual compounds. Different solvent systems will be used to elute compounds from column. Fractions collected from column will be monitored using TLC to identify flavonoid rich fractions.

Isolation and Purification of Flavonoids

Fractions showing similar TLC pattern will be combined and further purified to obtain isolated flavonoid compounds. Purity of isolated compounds will be confirmed by chromatographic techniques.

Quantitative Estimation of Flavonoids

The total flavonoid content present in the hydroalcoholic extracts of Euphorbia neriifolia leaves was determined by aluminum chloride colorimetric method.

Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) was employed as a simple, rapid, and reliable analytical technique for separation and identification of flavonoid compounds present in the isolated fractions.

Column chromatography fractions and observations

S. No	Mobile phase	Ratio	Purpose
1	Ethyl acetate: Formic acid: Acetic acid: Water	100: 11: 11: 27	Flavonoid separation
2	Chloroform: Methanol	9: 1	Alternative system

Characterization of isolated Compound

High Performance Thin Layer Chromatography (HPTLC) Analysis of Flavonoids

The isolated flavonoid extract (5-10 mg) was dissolved in HPLC grade methanol and sonicated for approximately 10 minutes to ensure complete dissolution. Standard flavonoid compound such as quercetin was prepared at known concentration. Both sample and standard solutions were filtered using membrane filter (0.45 µm) to remove particulate matter before application.

Pre-coa d silica gel 60 F254 aluminum plates were used as stationary phase. Samples were applied as narrow bands using CAMAG Linomat applicator at constant distance from bottom edge. The mobile phase consisting of ethyl acetate, formic acid, acetic acid, and water (100:11:11:26) was used for effective separation of flavonoids. The developed chromatogram was derivatized using 1% aluminum chloride solution in methanol and observed under UV light at 366 nm. Flavonoid compounds appeared as yellow fluorescent bands. Densitometric scanning was carried out using TLC scanner at appropriate wavelength to record peak area and Rf values. The concentration of flavonoids present in sample was estimated using calibration curve prepared from standard quercetin solution. The chromatographic profile provided fingerprint characterization of flavonoid compounds present in plant extracts [5-6].

Spectral Characterization of Isolated Flavonoid Compounds

Spectral characterization techniques were used to confirm the chemical structure and functional groups present in isolated flavonoid compounds. The purified compounds were dried thoroughly to remove moisture and residual solvent before analysis. Appropriate solvents were used depending on analytical technique employed.

UV-Visible Spectroscopy

UV-Visible spectroscopy was performed to study electronic transitions occurring in flavonoid molecules. A dilute solution of isolated compound was prepared in methanol and scanned over wavelength range of 200-600 nm. Flavonoids typically show two characteristic absorption bands: Band I in the range of 300-400 nm corresponding to cinnamoyl system of B-ring, and Band II in the range of 240-280 nm corresponding to benzoyl system of A-ring. Comparison of absorption maxima with standard compounds such as quercetin helped in identification of flavonoid structure [7-8].

RESULT & DISUSSION

Many plants include a broad class of polyphenolic compounds called flavonoids. They are widely recognized for their critical role in managing human and plant health. A wide spectrum of pharmacological functions, including anti-inflammatory, antibacterial, antiviral, anticancer, and cardioprotective properties, are exhibited by flavonoids. Because they combat free radicals, flavonoids are also beneficial to your health. Because they are so potent antioxidants, they may eliminate free radicals, reduce oxidative stress, and shield cells from the damage caused by chronic illnesses like cancer, diabetes, and heart disease. The health advantages of flavonoids are further enhanced by the possibility that they alter the function of enzymes, genes, and signaling pathways. Higher polarity solvents such as petroleum ether, chloroform, ethyl acetate, ethanol, and hydroalcohol (70% ethanol:water) allowed us to extract plant material from Euphorbia neriifolia leaves. Euphorbia neriifolia yielded 2.3% of petroleum ether, 3.6% of chloroform, 4.8% of ethyl acetate, and 7.4% of hydroalcohol. These findings unequivocally demonstrate that the maximum proportion of plant extract was obtained using hydroalcoholic extraction. Table 1 and Figure 1 presented the findings. Table 2 shows the results of the initial phytochemical screening. The ethyl acetate fraction's components including flavonoid compounds were separated using column chromatography. To ascertain their chemical composition, the flavonoid molecules that were separated from Euphorbia neriifolia underwent thorough spectrum analysis. Flavonoid chemicals were first identified using chromatographic methods including Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC). Spectroscopic methods, such as UV-visible spectroscopy, were used to validate the chemical structure further. During column chromatographic separation, compound Cl was extracted from the ethyl acetate fraction of Euphorbia neriifolia leaves. The presence of a flavonoid-type compound was suggested by the isolated chemical's appearance as an amorphous solid with a yellow color. To ascertain the compound's chemical structure, chromatographic and spectroscopic characterisation were performed. Compound Cl's thin layer chromatography revealed a clear spot with an Rf value of 0.32. The formed plate was sprayed with an aluminum chloride reagent and then exposed to UV light at 366 nm. The existence of a flavonoid nucleus was shown by the compound's intense yellow fluorescence. The isolated molecule's chromatographic performance verified that it is a moderately polar phenolic compound (Fig. 2). Compound Cl's HPTLC examination revealed a well-resolved peak at Rf value 0.34 ±0.02. The isolated

compound's purity was shown by a strong peak on the chromatogram (fig. 3). Compound C1's UV examination revealed λmax at 255 nm and 370 nm, respectively, confirming the existence of conjugated cinnamoyl and aromatic benzoyl systems, which unmistakably suggest the presence of flavonoid structure (fig. 4). The aforementioned discovery led to the identification of molecule C1 as quercetin, a flavanol compound with conjugated aromatic rings and a phenolic hydroxyl group.

Table 1: Percentage yield of successive solvent extracts of Euphorbia neriifolia leaves

S. No.	Extract	% Yield (w/w)
1.	PEEENL	2.3
2.	CEENL	3.6
3.	EAEENL	4.8
4.	HAEENL	7.4

PEEENL- Petroleum ether extract of Euphorbia neriifolia leaves CEENL - Chloroform extract of Euphorbia neriifolia leaves EAEENL - Ethyl acetate extract of Euphorbia neriifolia leaves HAEENL - Hydroalcoholic extract of Euphorbia neriifolia leaves

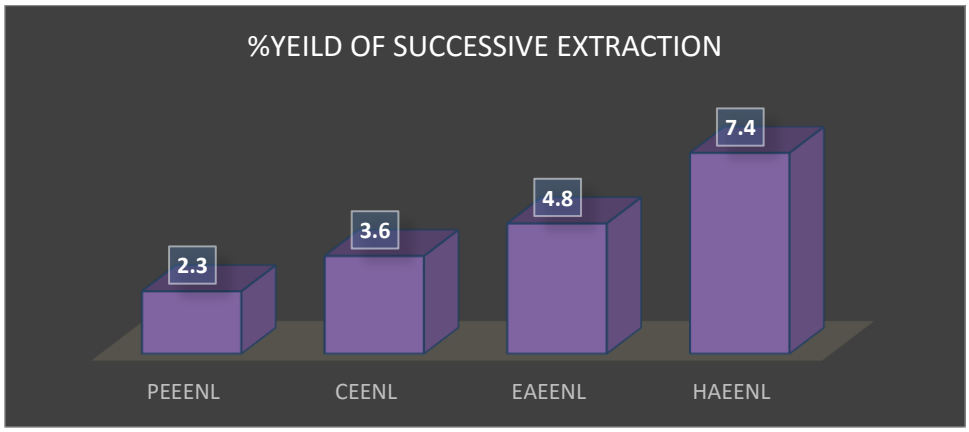

Fig. 1: Percentage yield of successive solvent extracts of Euphorbia neriifolia leaves

Table 2: Phytochemical Screening of Euphorbia neriifolia leaves extract

Test	PEEENL	CEENL	EAEENL	HAEENL
Carbohydrate	-	-	-	+
Anthraquinone	-	-	-	+
Terpenoid			-	-
Flavonoid	+	+	+	+
Saponin	-	-	-	+
Alkaloid	-	+	-	+
Tannin	-	-	+	+

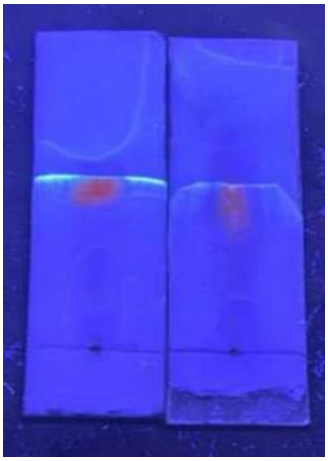

Fig. 2: TLC chromatogram of compound Cl

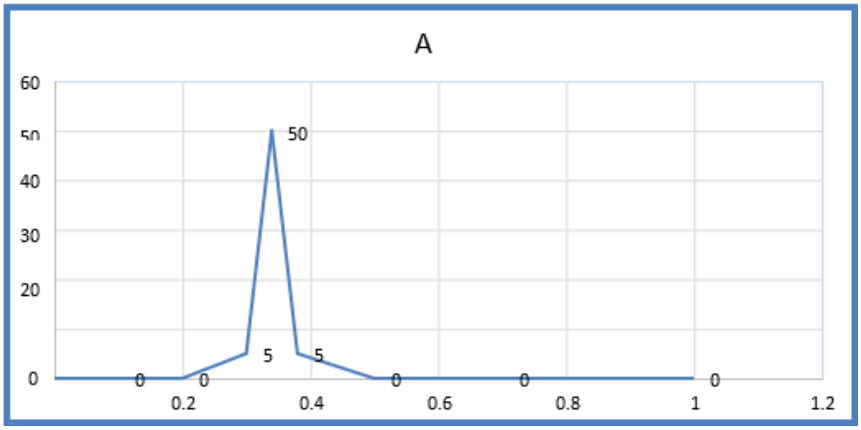

Fig. 3: HPTLC chromatogram of C1

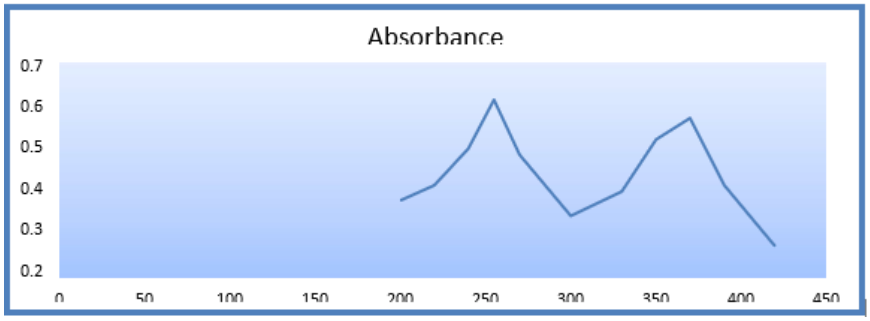

Fig. 4: UV Spectral of C1 Compound

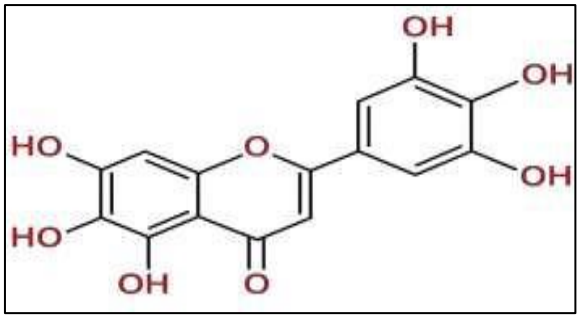

Fig. 5: Isolated compound C1(Quercetin)

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