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Isolation, Characterization and Phytochemical Evaluation of Active Compound Coumarin from *Oldenlandia corymbosa* (Linn)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Medicinal plants have served through ages, as a constant source of medicaments for the exposure of a variety of diseases. Plants are known to provide cures for various human illnesses and are a rich source of phyto constituents having diversified pharmacological properties. *Oldenlandia corymbosa* (L.) (syn. Hedyotis corymbosa Lam., Thai name Ya-Lin-Ngu), a member of the family Rubiaceae, is widely distributed in tropical regions of Asia. The decoction of whole plants is used in traditional medicine for antipyretic purposes to decrease body temperature. Pharmacologically, the anti-inflammatory, antioxidant, and hepatoprotective properties of plant extracts have been reported. This plant is well known to contain mainly iridoid glucosides. The whole plant contains reducing sugars, amino acids, flavonoids, steroids, glycosides and alkaloids. The present aim of the study is to extraction and isolation of bioactive compound from *Oldenlandia corymbosa* (L.). The isolation and characterization analysis included, Thin Layer Chromatography, High Performance Thin Layer Chromatography, GC-MS and Spectroscopy studies (IR, NMR, and MASS). The presences of coumarin in the methanolic fraction revealed that the isolated constituent is most active compound. Isolation of bioactive compound coumarin may help in identification of

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various pharmacological activities and carrying out further research in *Oldenlandia corymbosa* (L.).

Keywords: Rubiaceae; Oldenlandia corymbosa; GC-MS; isolation; characterization; coumarin.

1. INTRODUCTION

Herbal medicine represents one of the most important fields of traditional medicine in India. A number of traditional medicinal plants have been used in folk medicine to treat a wide range of human physical ailments [1,2]. The traditional medicines all over the world are nowadays reevaluated by an extensive activity of research on different plant species and their therapeutic principles (WHO 2002). Oldenlandia is a genus of flowering plants in the family Rubiaceae. It is pantropical in distribution and has about 240 species [3]. The type species for the genus is *Oldenlandia corymbosa* [4]. Oldenlandia was named by Linnaeus in 1753 in Species Plantarum [5]. The name honors the Danish botanist Henrik Bernard Oldenland (c.1663-1699) [6]. Some species are important in ethnomedicine; a number (usually island endemics) are threatened species, with one species and one variety being completely extinct already [7].

Some botanists have not recognized Oldenlandia, but have placed some or its entire species in a broadly defined Hedyotis [8]. More recently, the circumscription of Hedyotis has been narrowed to a monophyletic group of about 115 species and no longer includes Oldenlandia [9]. The genus Oldenlandia, as presently defined, is several times polyphyletic and will eventually be reduced to a group of species closely related to the type species. This group, known informally as Oldenlandia sensu stricto, is sister to a section of Kohautia that will eventually be separated from Kohautia and named as a new genus [10].

It is an annual herb with numerous stem, leaves subsessile, linear or linear-lanceolate, margins recurved and scabrous, stipules with bristles, flowers on filliform pedicles usually 2-3 cm long, lobes acute, fruit capsule, globose or pyriform, seeds are pale brown, angular, germinate at high temperature [11]. Oldenlandia, an herb prevalent in East Asia and Southern China, is used in traditional Chinese medicine to clear “heat” and to eliminate “toxins”. It is used in combination with other herbs for the treatment of hepatitis, snake bites, and tumors of the liver, lung, stomach and rectum [12,13].

Chart 1: Scientific classification of Oldenlandia [14]

Kingdom:	Plantae
Clade:	Tracheophytes
Order:	Gentianales
Family:	Rubiaceae
Subfamily:	Rubioideae
Tribe:	Spermacoceae
Genus:	Oldenlandia

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The fresh sample leaves of *Oldenlandia corymbosa* linn were collected from their natural habitat near Sasthamcotta, Kollam district, Kerala [15]. Samples were authenticated (PHARM 17 BO3) by the pharmacognosist and the voucher specimens were kept in Lab of Dept. of Aromatic and medicinal plants research station,odakkali Kerala agricultural University, Kerala. They were shade dried and packed in zip lock polythene bag and labeled.

Fig. 1. Oldenlandia plant extract

2.2 Extraction

The collected plant materials were washed twice in running tap water and shade dried at room temperature for 3 weeks. The air-dried plant leaves were pulverized, using an electric blender to make a fine powder. A total of 3 kg of powdered *Oldenlandia corymbosa* linn leaves

was sequentially extracted with methanol using a Soxhlet apparatus until the efflux solvents become colorless. The after Extraction the extract passed through the Whatman filter Paper (Whatman No. 1) to avoid impurities and dried under vacuum at 40◦C. The dried crude methanol extract was stored in a freezer at −4◦C for further study [16].

2.3 Separation of Active Compounds by Chromatography (Thin layer chromatography)

The column is to be prepared by plugging the lower part of the column with small amount of pre-extracted quartz wool (or glass wool), and by running with non-polar solvent mixture. This step is to be followed by addition of activated silica gel (or alumina) to the column to the required volume. Finally, extractable organic matter of the sample to be fractionated has to place on top of the column. Plugging the lower part of the column with small amount of or glass wool), and by rinsing with polar solvent mixture. This step is to be followed by addition of activated silica gel (or alumina) to the column to the required volume. Finally, extractable organic matter of the sample to be fractionated has to be placed on top of the column.

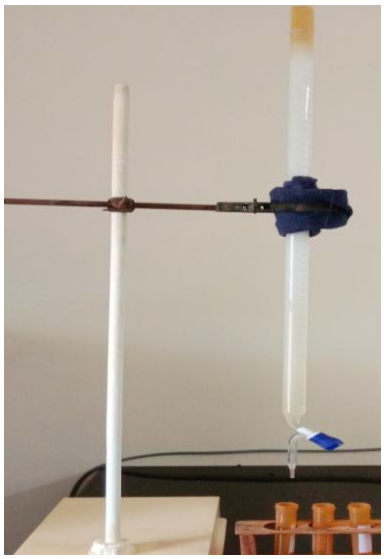

Fig. 2. Column packing

The residue was chromatographed on silica gel preparative slides using different solvent systems: acetone, hexane and ethyl acetate, separately, or combined solvent systems; acetone:ethyl acetate (1:2), acetone:ethyl acetate. (2:1), hexane: ethyl acetate (1:2), and hexane: ethyl acetate (2:1). The starting crude spots were observed for migration and separation by the previously prepared mobile phases. R values of the obtaining colored and non-colored spots with the aid of visible and UV lamps were recorded. Using silica gel plates (20· 20 cm dimensions and 0.50 mm thickness of 60GF254 fine grade), the active bands were gathered, dissolved in ethyl acetate and concentrated to dryness in the vacuum. The dried TLC plate was viewed by iodine vapor and visualized under UV light (low and high wavelength) [17].

The culture broth (10 L) was extracted with ethyl acetate (1:1v/v) stepwise and concentrated by rotary evaporator at 50°C to yield 2 g of brown crude residue. The residue was chromatographed on silica gel preparative slides using different solvent systems: acetone, hexane and ethyl acetate, separately, or combined solvent systems; acetone: ethyl acetate (1:2), acetone: ethyl acetate. (2:1), hexane: ethyl acetate (1:2), and hexane: ethyl acetate (2:1). The starting crude spots were observed for migration and separation by the previously prepared mobile phases. R values of the obtaining colored and non-colored spots with the aid of visible and UV lamps were recorded. Using silica gel plates (20· 20 cm dimensions and 0.50 mm thickness of 60GF254 fine grade), the active bands were gathered, dissolved in ethyl acetate and concentrated to dryness in the vacuum [18]. The methanol extract of each fraction was purified and analyzed by GC–MS and HPLC. The structure of isolated compound was characterized by spectral studies UV, FT-IR, NMR and MASS studies.

2.4 Gas Chromatography–mass Spectrum [19, 20]

The active fraction was analyzed using the SHIMADZU GC–MS-QP5050A with program CLASS 5000 in the Central Lab facility. Identification was performed using WILEY MASS SPECTRAL DATA BASE Library.

2.5 Infrared [21]

One mg sample of extracted crude was subjected to IR-spectral analyses using Infrared Spectrophotometer. Mid IR region of 400–4000 cm-1 was used for sample analysis. A mixture of spectroscopic pure KBr was in the ratio of 5:95; pellets were fixed in sample holder.

3. RESULTS AND DISCUSSION

3.1 Thin Layer Chromatography Separation

Separation of the compounds standards by TLC by methanol extract of O. corymbosa was dissolved in 50 mL of hot water and extracted three times with 50 mL of ethyl ether and ethyl acetate, respectively. Solvents from all of the fractions were removed with a rotary evaporator to obtain the ethyl ether and ethyl acetate extract. The ethyl ether extract was separated by TLC (normal-phase plates, ethyl ether– hexane, 1:5, v/v), and the nine zones found on the TLC plate could be visualized under UV light at 254 nm. Each zone was scraped from the plate and extracted with methanol. The methanol extract of each fraction was analyzed by GC–MS and HPLC.

Fig. 3. TLC of coumarin

Based on the Rf values of the bands the active principles were identified with standard. Under UV light – for chromone system, TLC of coumarin visualized with UV light) shows spots, and based on relative Rf values, After elution, the purity of each fraction was tested by analytical TLC which showed clear separation of fractions. This fraction was scraped and collected (Fig.4) for further analysis.

3.2 GC-MS Analysis

In GC –MS Analysis there are major predominant bioactive compound identified and named as Coumarin, where the sample was passed through single pole GC analysis instrument and further the extracted bioactive compound are focused and subjected to isolation ,purification procedure and the structure of isolated compound Coumarin was identified by spectral studies [20].

3.3 HPLC Analysis

The purity of isolated compounds was checked by HPLC analysis and spectra recorded and given in Fig.6. The isolated active compound shows a separation peak at a retention time of 11.37 min for active compounds which is compared with standard respectively. The purity of the active compounds (coumarin) was indicated as a single sharp peak.

3.4 UV- spectrum Analysis of Isolated Compound

As per the reference the UV visible spectrum (Fig. 7) analysis the isolated fraction of Coumarin shows 278 nm peak, confirmed as Coumarin with the reference [22].

3.5 IR spec of Coumarin

IR spectrum (Fig.8) of purified Coumarin shows many peaks corresponding to functional groups which are present in structure of isolated compound there were a broad peak at 3173.71 cm−1, which corresponds to the Hydroxyl group (O-H). The aromatic C–H stretching peak was observed at 3031.78 cm−1 while aliphatic C-H stretching was observed in 2886.89 cm−1, C=C stretching frequency was found at 1796.92 cm−1. The absorption band at 1156.81 and 1021.33 cm−1 corresponds to the C-C stretch of Benzene ring and C-O bend, respectively [22].

3.6 NMR- spectrum Analysis of Isolated Compound

From the 1H NMR spectrum (Fig.8) of the isolated Coumarin, spectrum consists of matching expected chemical shifts to the expected moieties. The chemical shifts were matched with the existed earlier reports. For coumarin, chemical shifts 103.326, 109.818 and 103.322 were characteristic (Fig. 8)

3.7 Mass of Coumarin

Through the Mass spectrometric analysis the isolated compound identified as Coumarin with the molecular weight of 146.22.Mass spectral analysis suggested MS at m = z 134 of compound. It was represented in this data that this compound corresponds to a molecular formula of $C_9H_6O_2$ [23].

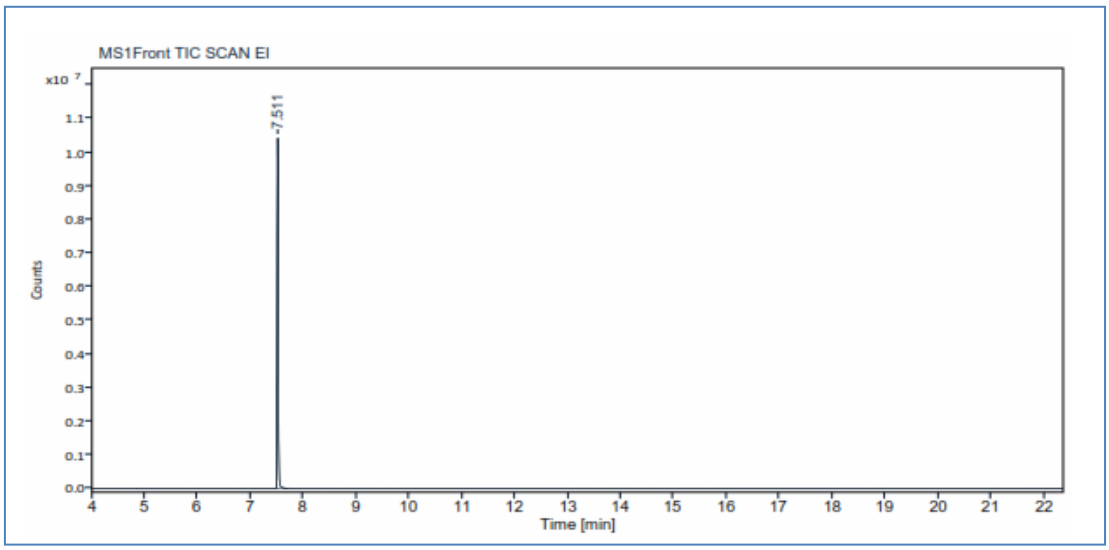

Fig. 4. GC-MS- analysis of Coumarin

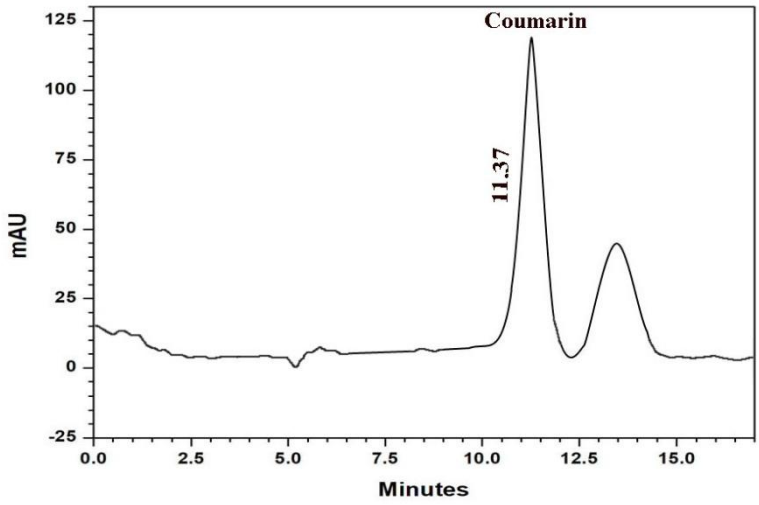

Fig. 5. HPLC (Coumarin Separation)

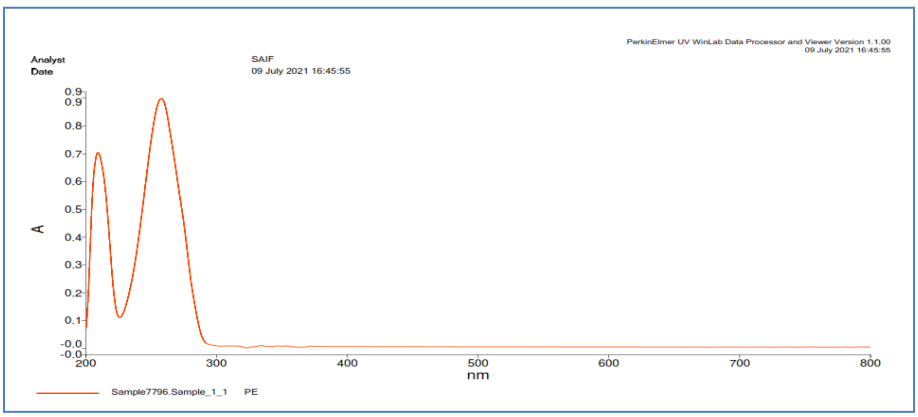

Fig. 6. UV-Vis spectrum of Coumarin

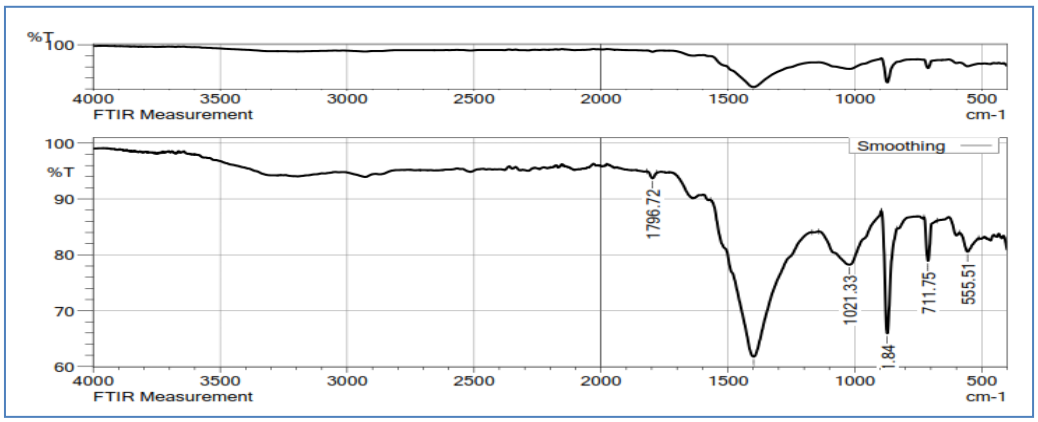

Fig. 7. IR- spectrum of Coumarin

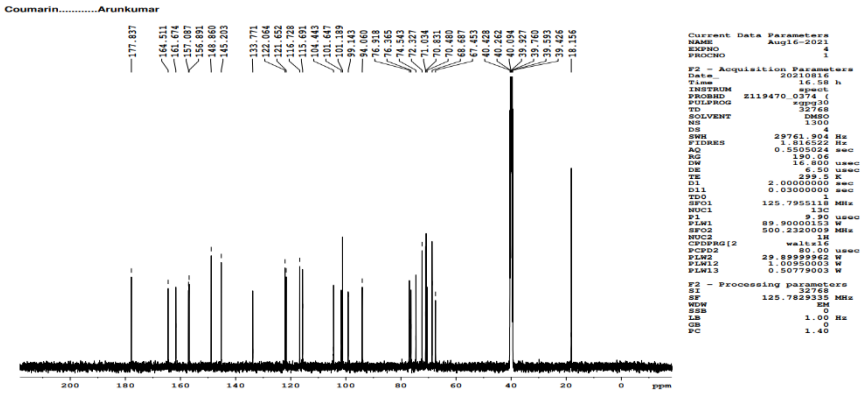

Fig. 8. NMR- spectrum of Coumarin

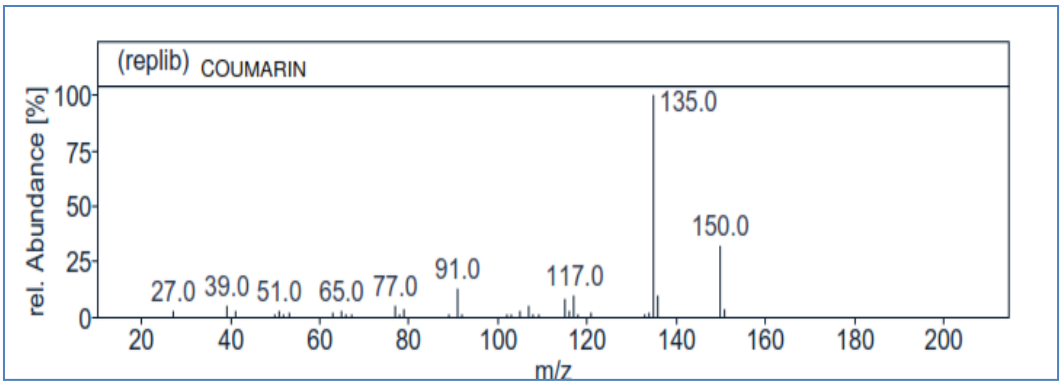

Fig. 9. Mass Spectrum of Coumarin

4. CONCLUSION

The bioactive compound was isolated by chromatographic techniques (TLC), Purified using HPLC and GC-MS analysis. Finally the structure of the compound was appraised by IR, NMR and MASS spectroscopy studies which revealed that the isolated compound is coumarin compare with the standard. From the above result conclude that the methanolic extract of plant O. corymbosa is good source of coumarin. Hence, this study recommends that the isolated active compound coumarin can be used as a prototype molecule for medicinal drug. The plant needs to be further evaluated in combination with other plants of same family to establish this

common weed as a pharmacologically potential herb.

RESEARCH SIGNIFICANCE

The study highlights the efficacy of "traditional medicine" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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