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Isolation and Identification of Flavonoid Compounds in *Tremaorientalis* Leaves by Preparative TLC and Various Spectroscopic Techniques

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Abstract

The increasing interest in powerful biological activity of secondary metabolites present in plants has shown necessity of determining their contents in medicinal plants. The present study is intended to find out the active constituent for *Trema orientalis* plant which is rarely mentioned in published books and there is no data of showing which constituents are present in this plant. Qualitative chemical examination of various successive extracts of leaves presence of phytosterols, carbohydrates, phenolic compounds, tannins, fixed oils and mucilage were present. Isocratic elution column chromatography was used for isolation of compounds. Isolation of flavonoids was done by preparative TLC, using ratio of chloroform: ethyl acetate (5:4) and four compounds were isolated. These four compounds gave shinoda test positive which indicates the presence of flavonoid. This was again conformed by TLC using Boric acid (3%) and oxalic acid (10%) spraying reagent. Spectroscopic analysis data gave the probability of flavones compound.

Keywords

Trema orientalis, Preparative TLC, Spectrophotometric methods like UV, IR, Flavone

Greentree Group

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1. INTRODUCTION

Trema orientalis which is popularly known as *Gol* in Ayurveda is distributed more or less throughout (except Kutch) in deciduous forests of India, Ceylon, China-Malay Island, Singapore, Australia, tropical and subtropical regions of Pakistan[1,2]. Leaf, root and stem of the plant are reported for treatment of diarrhea[3], passing of blood in the urine, epilepsy and muscular pain[4]. Literature revealed that phytopharmacognostical parameters have not been reported for the leaf of this plant. Therefore, the main aim of the present work is to isolate and identify active constituent from leaf of *Trema orientalis* which could be used to explore this plant.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Fresh leaves were collected from the Idar, Gujarat during November, 2008. Botanical identification was carried out using local floras and authenticated by Dr. Reddy, Prof. and Head of Botany Dept., Sardar Patel University, Vallabh Vidyanagar. Voucher herbarium specimen [HSP/TO-6/37] is preserved along with crude drug sample at the herbarium of A. R. College of Pharmacy, Vallabh Vidyanagar.

2.2 Isolation of phytoconstituents by preparative TLC[5-7]:

Methanol extract was selected for isolation of the compound. The isolation was done by preparative TLC. In this substance of interest was scrapped from the layer after detection and subsequently examining it. Isolation of flavonoids was done by column chromatographic technique, using ratio of chloroform: ethyl acetate (5:4) and four compounds were isolated by suitable analytical technique. The mobile phase used was chloroform: ethyl acetate (5:4). After spotting and developing the plate in the solvent system it was dried and then observed in day light. Four spots at R_f value 0.27, 0.459, 0.797, 0.972 were observed. These spots were scrapped and placed in petridish. Methanol was added to it and then the mixture was filtered. The filtrate was evaporated to obtain four samples. The samples were then analyzed in UV-Visible Spectrophotometer for λ max determination and FTIR studies for identification.

2.3 Test for flavonoids [8-10]

Shinoda Test: Four pieces of magnesium fillings (ribbon) were added to the ethanolic extract followed by a few drops of concentrated hydrochloric acid. A reddish colour indicates the presence of flavonoid.

2.4 Spectroscopic Studies: [11-12]

2.4.1 U.V. Spectroscopy

Make: Perkin Elmer, U.S.A.

Model: Lambda 19

Specification: Double beam double Monochromator, Ratio Recording

Lamp: Deuterium (UV), Tungsten-Halogen (VIS/NIR)

Detector: Photomultiplier (UV/VIS), Lead sulphide cell (NIR)

Wavelength: 185-3200 nm

Scan speed: 0.3 to 1200 nm/min

Wavelength Accuracy: ±0.15 nm for UV/VI ±0.15 nm for NIR

Baseline flatness: ± 0.001 A, 4 nm, slit, Golay Savitzky smooth Q

Ordinate mode: Scan, Time drive, Wavelength, Programme, Concentration

Photometric Accuracy: ±0.003 A or ±0.08

2.4.2 FTIR Spectroscopy

Make: Perkin Elmer, U.S.A.

Model: Spectrum GX-FTIR

Scan Range: 15600 cm^{-1} -30 cm^{-1}

Scan time: 20 scan/second

Resolution: 0.15cm^{-1}

Single Beam/Ratio: Single

Detector: MIRTGS & FIRTGC

Source NIR: 15000-1200 cm^{-1}

Beam splitter opt KBr: 7800-370 cm^{-1}

Detector MIRTGS: 10000-220 cm^{-1}

Optimum range: 7800-1200 cm^{-1}

Detector FIRTGS: 30-780 cm^{-1}

OPD velocity: 0.20 cm/s

Interferogram direction: Bi-direction

3. RESULTS

3.1 Preparative TLC

Four compounds S_1(R_f 0.27), S_2(R_f 0.45), S_3(R_f 0.79), S_4(R_f 0.97) were isolated by preparative TLC using silica gel G as absorbent. The solvent system used was chloroform: ethyl acetate (5:4)

3.2 Spectroscopic Data

3.2.1 U.V Spectroscopy

The UV Spectra of isolated compound was obtained using Perkin Elmer UV Spectrometer. The Spectra was obtained 200-400 nm range. Λmax: 259.57nm. Data shown in figure 1 to 8.

3.2.2 IR Spectroscopy

The IR Spectra of isolated compound was obtained using Perkin Elmer IR Spectrometer. The Spectra was obtained 15600 cm^{-1} -30 cm^{-1} range. Data shown in figure 8-12 and table 1 to 4.

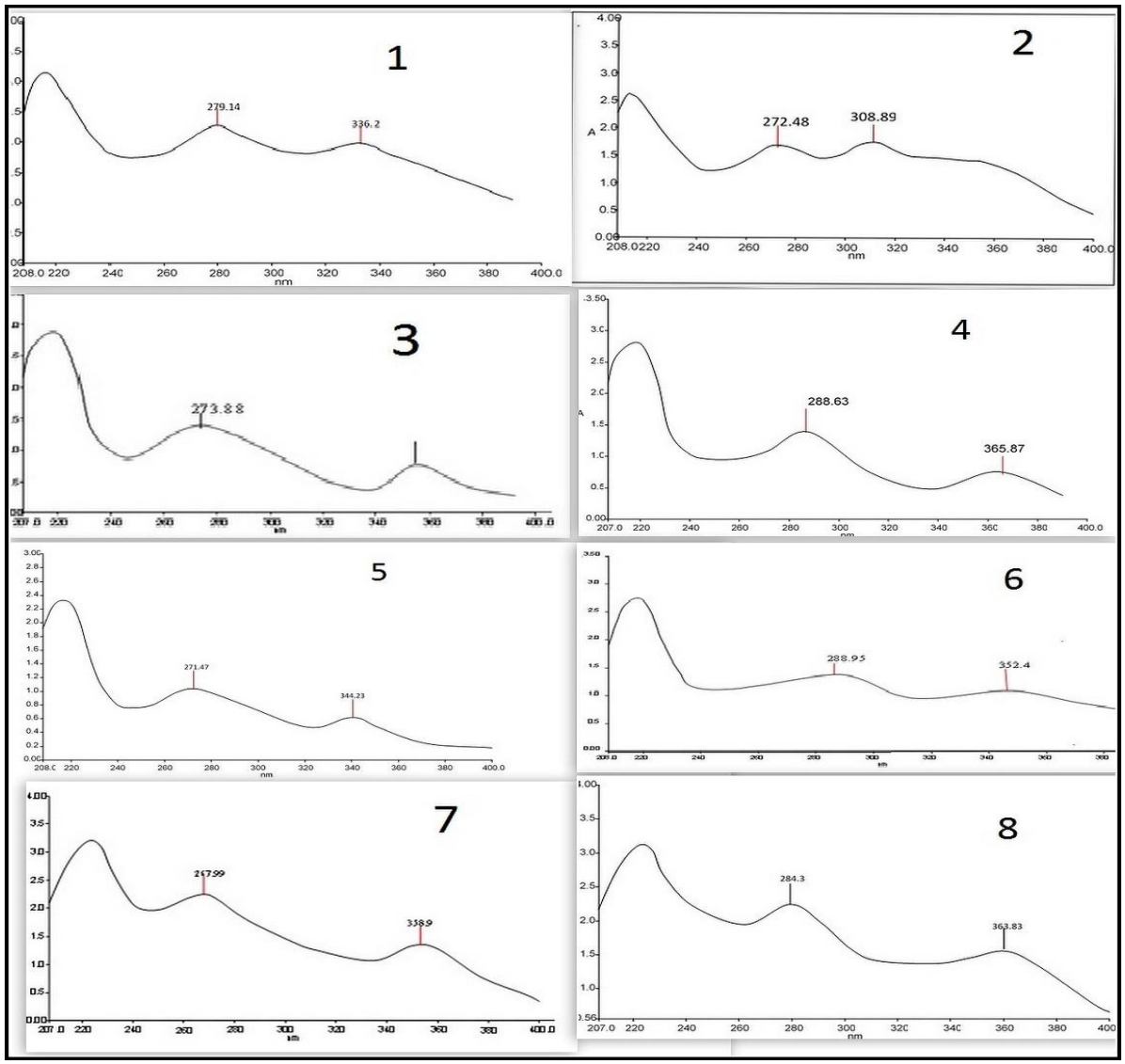

Figure 1 U. V. spectrum of isolated compound in ethanol of sample 1

Figure 2 U. V. spectrum of isolated compound in ethanol after addition of alkali of sample 1

Figure 3 U. V. spectrum of isolated compound in ethanol of sample 2

Figure 4 U. V. spectrum of isolated compound in ethanol after addition of alkali of sample 2

Figure 5 U. V. spectrum of isolated compound in ethanol of sample 3

Figure 6 U. V. spectrum of isolated compound in ethanol after addition of alkali of sample 3

Figure 7 U. V. spectrum of isolated compound in ethanol of sample 4

Figure 8 U. V. spectrum of isolated compound in ethanol after addition of alkali of sample 4

Table 1 IR spectra of sample 1

Sr. no.	Wave numbers (cm^{-1})	Standard Wave no. range (cm^{-1})	Characteristics
1	2922.4	2850-2960	C-H Stretching(alkane)
2	2852.60	2850-2960	C-H Stretching(alkane)
3	2095.94	2100-2200	C=C Stretching (alkene)
4	1734.56	1735-1750	C=O Stretching (ester)
5	1462.19	1450-1600	C=C Stretching(Aromatic)
6	1362.30	1310-1410	C-O Stretching(Phenols)

Table 2 IR spectra of sample

Sr. no.	Wave numbers(cm^{-1})	Standard Wave no. Range (cm^{-1})	Characteristics
1	2924	2850-2960	C-H Stretching(alkane)
2	2853.22	2850-2960	C-H Stretching(alkane)
3	2083.52	2050-2200	C=C Stretching (alkene)
4	1509.11	1450-1600	C=O Stretching (aromatic)
5	1458.38	1450-1600	C=C Stretching(Aromatic)
6	1365.22	1310-1410	C-O Stretching(Phenols)

Table 3 IR spectra of sample 3

Sr. no.	Wave numbers (cm^{-1})	Standard Wave no. range(cm^{-1})	Characteristics
1	2924	2850-2960	C-H Stretching(alkane)
2	2853.45	2850-2960	C-H Stretching(alkane)
3	2083.27	2050-2200	C=C Stretching (alkene)
4	1713.52	1705-1725	C=O) stretching (Ketone
4	1513.28	1450-1600	C=O Stretching (aromatic)
5	1456.43	1450-1600	C=C Stretching(Aromatic)

Table 4 IR spectra of sample 4

Sr. no.	Wave no. (cm^{-1})	Standard Wave no. range(cm^{-1})	Characteristics
1	2924.93	2850-2960	C-H Stretching(alkane)
2	2856.08	2850-2960	C-H Stretching(alkane)
3	2081.18	2050-2200	C=C Stretching (alkene)
4	1509.67	1450-1600	C=O Stretching (aromatic)

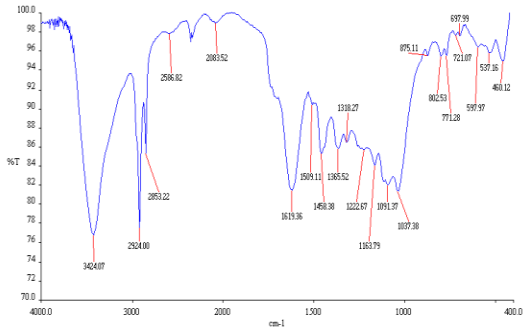

Figure 9 IR spectra of Sample 1

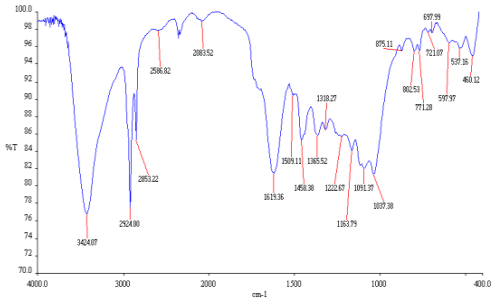

Figure 10 IR spectra of sample 2

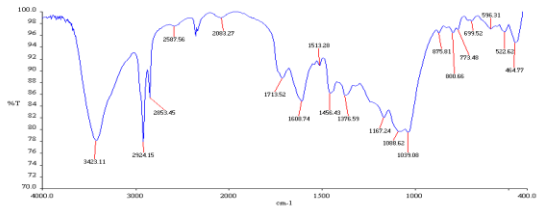

Figure 11 IR spectra of sample 3

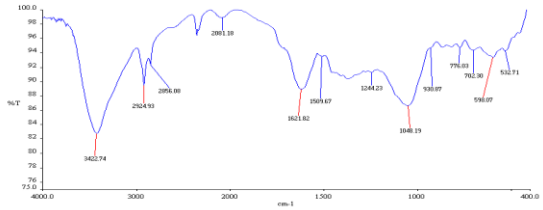

Figure 12 IR spectra of sample 4

DISCUSSION

Plants and other natural products have been in use since ages for health and maintenance of life. The Vedic literature, the most authentic, ancient Indian scripture, gives the reference of many plants for different diseases and their prevention.

Based on the report of Physicochemical Screening of Leaves extract, the methanol extract contain flavonoid. Isolation of flavonoid was done by Preparative chromatographic technique, using Chloroform: ethyl acetate (5:4) and four compounds were isolated. These compounds were flavonoids which are confirmed by chemical test and TLC with Boric Acid (3%) and Oxalic acid (10%) reagent. Spectroscopic data of UV, IR also confirm the structure of flavonoid, it may be flavone (apigenin type of aglycone).

CONCLUSION

Qualitative chemical examination of various successive extracts of leaves showed the presence of phyosterols, triterpenoids, flavanoids, fixed oil, tannins and carbohydrates. Four compounds were isolated from column chromatographic technique and further they are confirmed as flavanoids by UV and IR spectroscopic techniques. HPTLC finger printing can be also become a useful tool for confirmation of flavonoids compounds.

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