

Flavan-3-ol Isomers Isolated from *Euphorbia Thymifolia* Linn

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ABSTRACT

Objective: Isolation and identification of phytoconstituents from methanolic extract of whole herb of *Euphorbia thymifolia* L. **Material and Methods:** Methanolic extract of *Euphorbia thymifolia* whole herb was prepared by hot soxhalation method and Flavan-3-ol isomers (compound (I) and (II)) were isolated using chromatographic techniques. The structural identification Flavan-3-ol was carried out by employing Infra red spectroscopy (IR) and ¹H Nuclear magnetic Resonance technique. Spectral data obtained was compared with the available literature. **Results and conclusion:** Spectral analysis revealed compound (I) and (II) as catechin and epicatechin respectively. These compounds have been reported for the first time in this plant

and can serve as a useful tool in its standardization.

Key words: *Euphorbia thymifolia*, Flavan-3-ol, Catechin, Epicatechin etc.

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INTRODUCTION

A numbers of bioactive compounds in medicinal plants, such as alkaloids, tannins, flavonoids, sterols, triterpenes, etc., are noted to play major role in physiology and management of diseases.¹ Flavonoids constitute one of the most exclusive classes of compounds in medicinal plants.² The foremost important task in this paradigm is the screening of flavonoids in plants. Chromatographic studies of these compounds serves to be a very useful and reliable source in the process of bioactive compounds screening in plants.³ According to the ethnobotanical information, it has been reported that the plant *Euphorbia thymifolia* (family-Euphorbiaceae) is a medicinal herb used traditionally in dysentery, bleeding piles, gonorrhoea, dysmenorrhoea, amenorrhoea, helminthiasis, ringworm, chronic cough, asthma, bronchitis, cardiac debility, greying of hairs, skin diseases etc.⁴⁻⁵ *Euphorbia thymifolia* have numerous pharmacological activities including antibacterial,⁶⁻⁷ antifungal,⁸ antimicrobial,⁹ anti-inflammatory,¹⁰⁻¹¹ antiviral,¹² antispasmodic, bronchodilator, antibronchial, asthmatic,¹³⁻¹⁵ hypoglycaemic,¹⁶⁻¹⁸ anticancer¹⁹⁻²⁰ and antioxidant.²¹

A number of chemical constituents are present in *Euphorbia thymifolia* whole herb and its different parts. According to the literature, the whole plant contains epitaraxerol, n-hexacosanol, euphorbol, 24-methylene cycloartenol, 12-deoxy-4P-hydroxyphorbol-13-dodecanoate-20-acetate, 12-deoxy-4P-hydroxyphorbol-13-phenylacetate-20-acetate, 12-deoxyphorbol-13,20-diacetate, quercetin-3P-galactoside, 12-deoxyphorbol-13,20-diacetate, 12-deoxy-4Phydroxyphorbol-13-dodecanoate-20-acetate, n-hexacosanol, esters, n-alkanes and sterols.²³⁻²⁴ Roots contain taraxerol and triucalol. Leaves and stems consist of 4-trihydroxy flavone-7-glycoside.²² Leaves contain ellagitannin dimers, euphorbins G and H with 12 known polyphenols.^{19, 23-28} The present work deals with the isolation and identification of compounds from methanolic extract of *Euphorbia thymifolia* whole herb.

MATERIALS AND METHODS

Plant Material

The fresh whole herb of *Euphorbia thymifolia* was collected from the Tau Devilal National Herbal Park, Khizrabad (Yamunanagar), Haryana, India and authenticated by Regional Research Institute, Bangalore, India.

A voucher specimen number RRCBI-MUS-126 has been stored at the facility.

Chemical

Silica gel 60-120 mesh (nice chemicals), chloroform A.R. (nice chemicals), ethyl acetate (nice chemicals), methanol (nice chemicals) and instruments includes UV chamber, ¹H nuclear magnetic resonance (¹H NMR) spectra were recorded on Bruker Avance II 400 NMR spectrometer in appropriate deuterated solvents. Chemical shifts were reported as δ (ppm) relative to tetramethylsilane (TMS) as internal standard. Infrared (IR) spectra were recorded as KBr pellet on Perkin Elmer FTIR spectrometer. The wave number is given in cm⁻¹.

Preparation of extract

Shade dried whole *Euphorbia thymifolia* was extracted by hot soxhlation using methanol. The extract was concentrated using vacuum rotary evaporator at 40°C and dried in a hot-air oven at 40°C. The extract was stored in a refrigerator at 4°C throughout the duration of study.

Qualitative estimation of phytoconstituents

The preliminary qualitative screening of methanol extract of *Euphorbia thymifolia* was carried out by employing various phytochemical tests.²⁹

Isolation of compounds

The isolated compounds from the methanolic extract of *Euphorbia thymifolia* were subjected to column chromatography. The column with internal diameter 2.5 cm and length 62 cm was packed with silica (60-120 mesh) using chloroform. The sample was packed with silica in ration 50:1. The column was equilibrated with pure chloroform. Initially run the column with pure chloroform and 1-20 fractions shows no compound elution confirmed with TLC. The compound was eluted isocratically using chloroform: ethyl acetate (98:2). The fractions collected were subjected to thin layer chromatography (TLC) with size 2x7 cm to check homogeneity of various fractions in the chloroform: ethyl acetate (99.9:0.1) solvent system. Chromatographically identical

fractions were collected in the same polarity solvent and evaporated to dryness. The concentrated fractions were purified by re-crystallization.³⁰ Elution of the column with chloroform: ethyl acetate (98:2) gave a yellowish powdery compound after crystallization from methanol.

Separation of isomers with preparative TLC

The eluted isomer compounds were recrystallized in methanol and subjected to separation with preparative TLC. The samples were applied on the preparative TLC and run in chloroform: ethyl acetate (99.5:0.5) (mobile phase) in glass chamber. After the successful development of TLC plate, the plate was examined under the UV Chamber at 254 nm and marked with graphite tip. The selected area was then scratched out along with the silica, with a sharp scalpel and collected in the glass tube. The scratched samples were dissolved in methanol along with silica, filtered and recrystallized. The compounds were air dried and weighed.³⁰ For identification of compound IR and ¹H-NMR analysis was carried out.

RESULTS AND DISCUSSION

The preliminary qualitative screening of methanol extract of *Euphorbia thymifolia* herb indicated the presence of flavonoids, phenolic, tannins, alkaloids, steroids, saponins and terpenoids

Two compounds were eluted simultaneously by the column chromatography and these compounds were identified by IR and NMR study. These two compounds were found to be isomers. These isomers were further separated by preparative TLC.

The weight of compound (I) and (II) was found 9.8 mg and 7.5 mg respectively. The description of IR and NMR peaks is given in (Table 1). By analysis of spectra and from previous literature available the structure of compounds was found as Flavan-3-ol isomers. Flavan-3-ols, such as epicatechin, catechin and their oligomers, represent a major class of secondary polyphenolic plant metabolites.³¹ Flavan-3-ols are mostly present in higher plants, and their high content in certain food plants, such as *Vitis vinifera*. Also a number of isomers isolated from plants can be easily separated with TLC_{prep}.^{3-4,32-33} In the present study, the compound (I) and (II) were found as catechin and epicatechin respectively, as per analysis of spectra and comparison with literature. The IR spectra

of compound (I) catechin has band around 3361-2891 cm⁻¹ region corresponding to aliphatic and aromatic C-H, phenolic and alcoholic O-H stretching. A band at 1619-1492 cm⁻¹ observed may be due to aromatic C=C. A band at 1400 cm⁻¹ represents the C-C stretching. The ¹H-NMR spectra of compound (I) was very clear and understandable. Catechin molecule contains 15 carbons. Aromatic carbons showed peaks at 7.08-6.93 (s, 3H), 6.75-5.51 (d, 2H), 5.93 (s, 4H, OH), 3.43 (s, 1H, OH) and other carbons at 2.49 (d, 2H, CH₂).

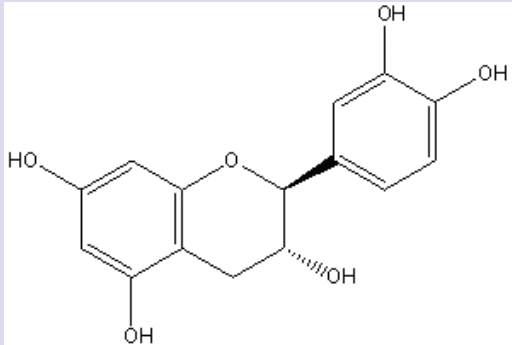
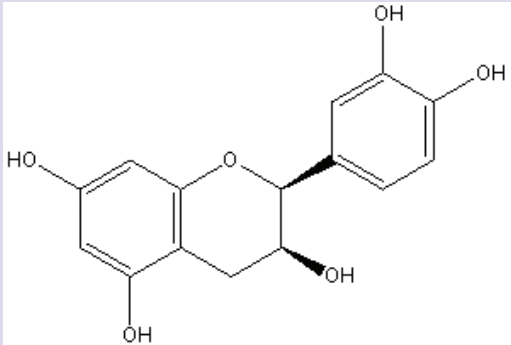
The IR spectra of compound (II) epicatechin showed band at 3628 cm⁻¹ due to O-H stretching and 3160 cm⁻¹ due to C-H stretching. A band at 1691 cm⁻¹ represents C=O stretching and 1247 cm⁻¹ represents C-O stretching. A band at 1645-1432 cm⁻¹ observed may be due to aromatic C=C stretching. The ¹H-NMR spectra of compound (I) epicatechin molecule contains 15 carbons. Aromatic carbons showed peaks at 8.44-7.90 (s, 3H), 7.74-47 (d, 2H), 5.06-4.20 (s, 4H, OH), 4.12 (s, 1H, OH) and other carbons at 2.50 (d, 2H, CH₂)

The catechins are the part of flavan-3-ols including (+)-catechin, (-)-epicatechin, (-)-ent-catechin, (+)-ent-epicatechin and their derivatives (C-3-O-esters).³⁴ Catechins are very common in vegetables and fresh fruit and important ingredients in our diet. Catechins possess numerous biological activities such as antimutagenic activity, antitumor activity and antioxidant properties.³⁵⁻³⁷ Due to antioxidant properties of catechin, plants exhibit protective effects against diseases involving oxidative stress such as cancers,³⁸⁻³⁹ cardiovascular diseases⁴⁰⁻⁴¹ and neurodegenerative diseases.⁴² (-)-Epicatechin also plays a major role in the improvement of blood flow for cardiac health.⁴³ The epimers (-)-ent-catechin, (-)-ent-gallocatechin, and their gallates are effective in inhibiting cholesterol absorption.⁴⁴

CONCLUSION

Flavan-3-ol isomers (compound I and II) were isolated and identified by column and thin layer chromatography from methanol extract of *Euphorbia thymifolia* Linn whole herb. The compound (I) was found as catechin (2, 3-*trans*-3, 4-*trans*flavan-3-ol) and compound (II) as epicatechin (2, 3-*cis*-3, 4-*trans*flavan-3-ol). These compounds have been reported for the first time in this plant and can serve as a useful tool in its standardization.

Table 1: IR and ¹H NMR spectroscopic data for compound (I) and compound (II) in CDCl₃

Compound	compound (I) <i>Trans</i> (ET1)	compound (II) <i>Cis</i> (ET2)
Weight	9.8 mg (pale yellow)	7.5 mg (off white)
IR	3628 (O-H stretching), 3100 (C-H stretching), 1688 (C=O stretching), 1619-1492 (C=C stretching), 1400 (C-C stretching), 1270 (C-O stretching).	3628 (O-H stretching), 3160 (C-H stretching), 1691 (C=O stretching), 1645-1432 (C=C stretching), 1400 (C-C stretching), 1247 (C-O stretching).
NMR	7.08-6.93 (s, 3H, aromatic), 6.75-5.51 (d, 2H, aromatic), 5.93 (s, 4H, OH, aromatic), 3.43 (s, 1H, OH, aromatic), 2.49 (d, 2H, CH ₂)	8.44-7.90 (s, 3H, aromatic), 7.74-47 (d, 2H, aromatic), 5.06-4.20 (s, 4H, OH, aromatic), 4.12 (s, 1H, OH, aromatic), 2.50 (d, 2H, CH ₂)
Name	Catechin	Epicatechin
Structure		

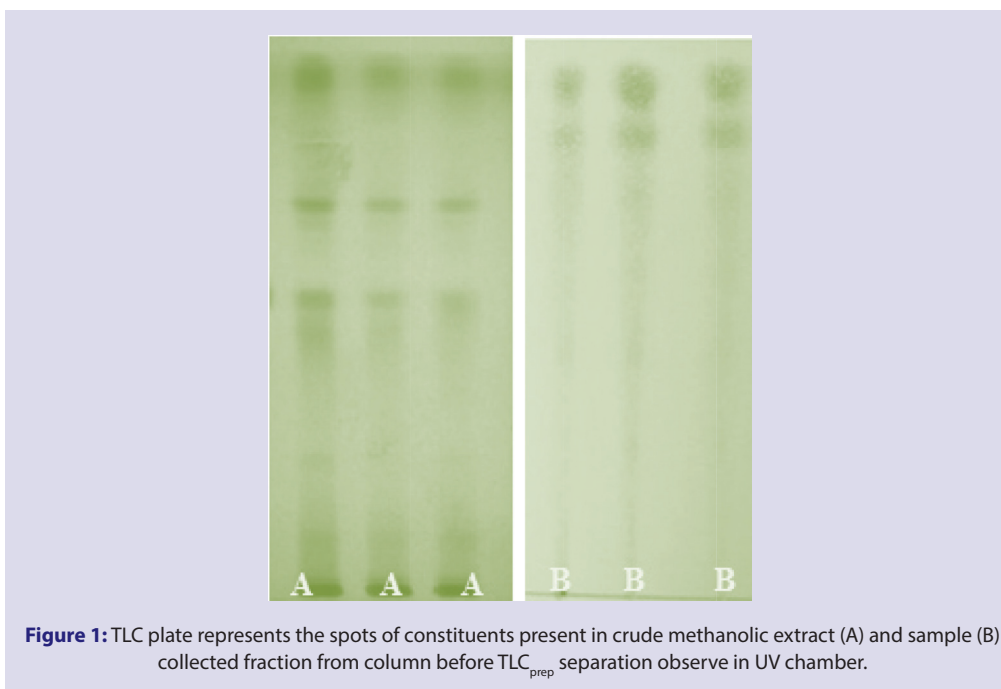
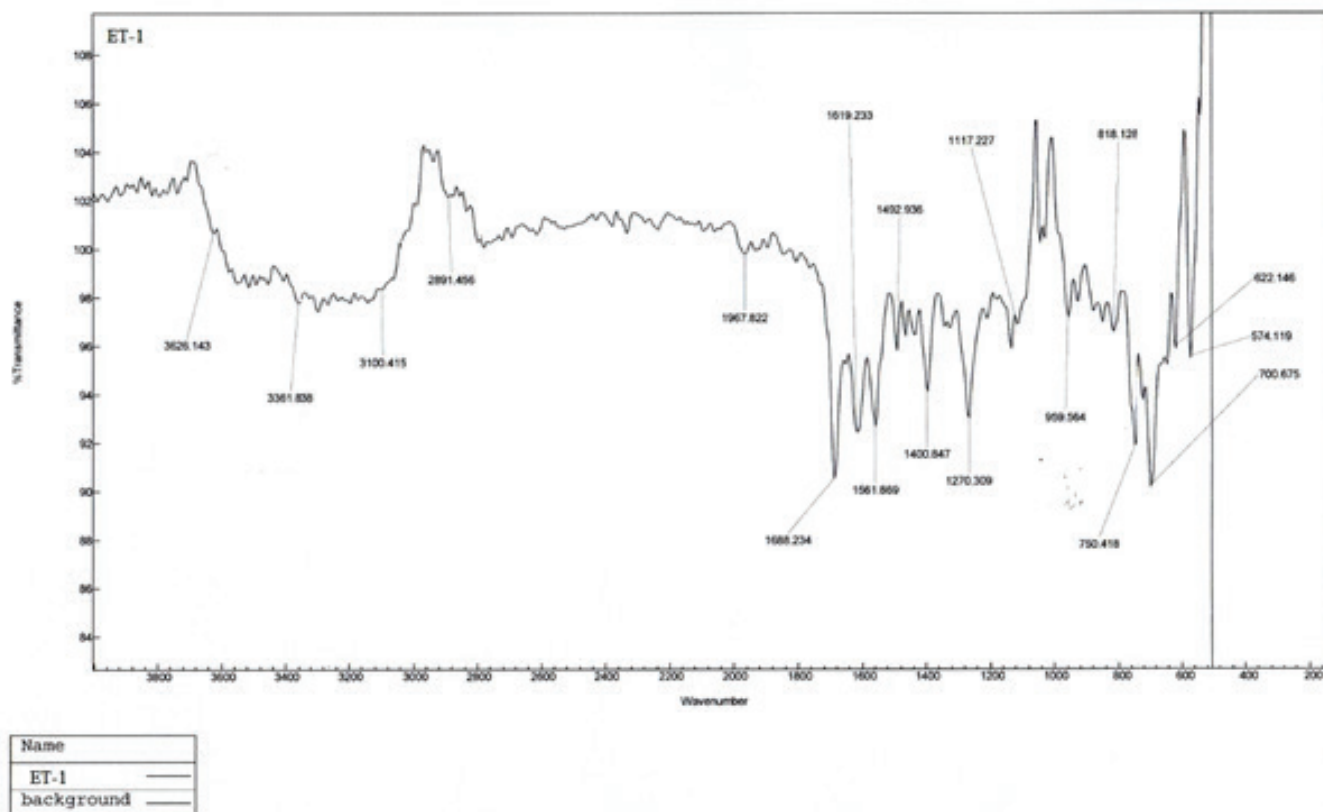
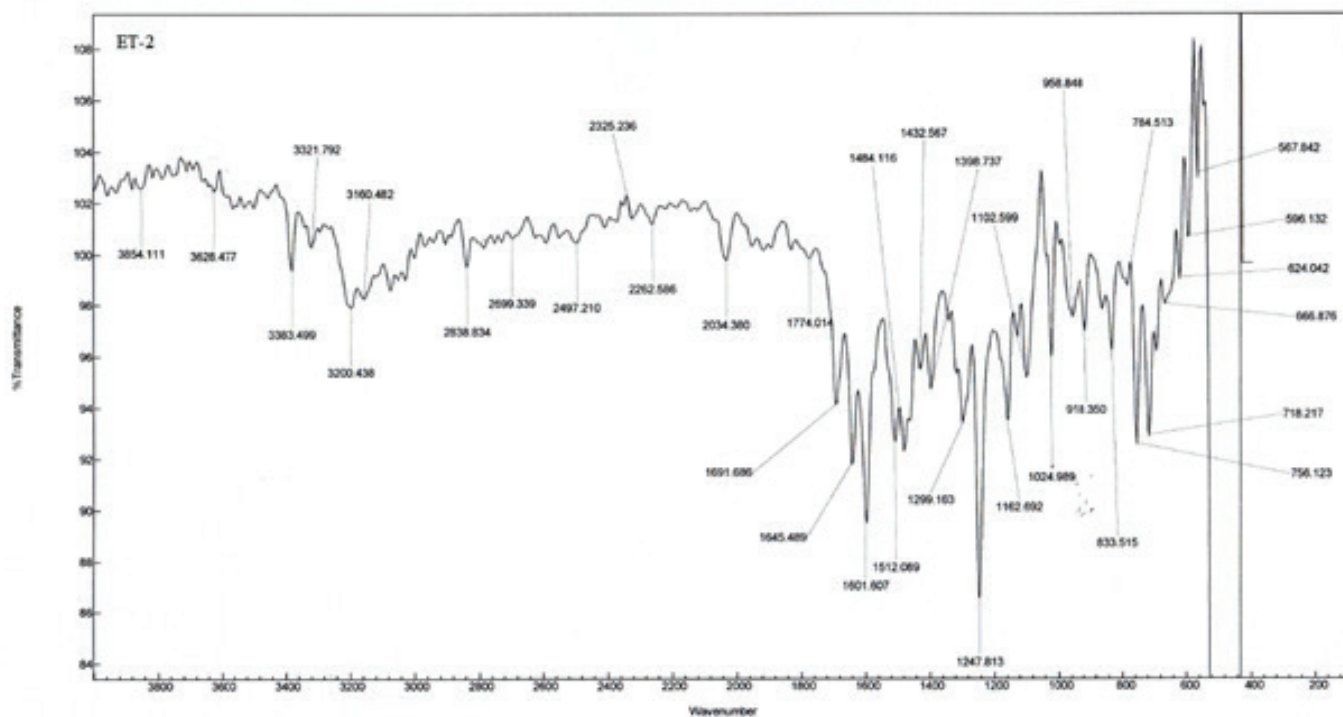


Figure 1: TLC plate represents the spots of constituents present in crude methanolic extract (A) and sample (B) collected fraction from column before TLC_{prep} separation observe in UV chamber.

IR spectra for ET1 (compound 1)

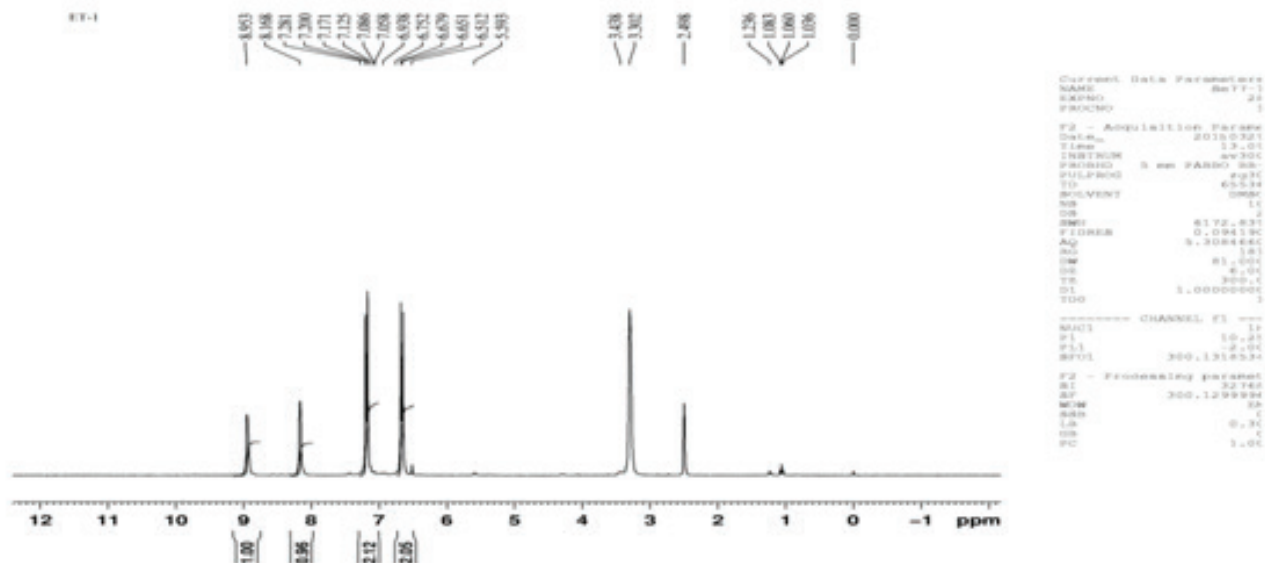


IR spectra for ET2 (compound II)

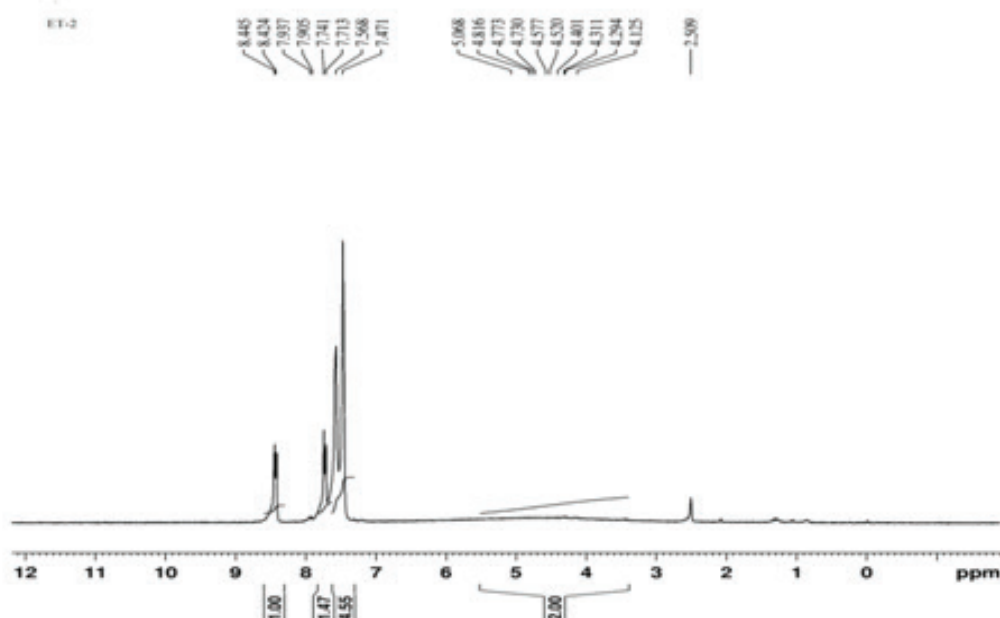


Name	
ET-2	—
background	—

NMR spectra for ET1 (compound 1)



NMR spectra for ET2 (compound II)



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PROCNO    1

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PULPROG  zgpg30
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RG       143.1
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F2 - Processing parameters
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WDW      EM
SSB      0
GB       0
PC       1.00

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATION USED

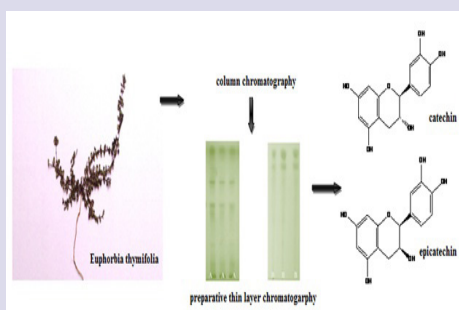
°C: Degree Centigrade; **¹H-NMR**: Proton Nuclear Magnetic Spectra; **IR**: Infra Red Spectra; **UV**: Ultraviolet; **TLC prep**: Preparative thin layer chromatography, **TLC**: Thin layer chromatography.

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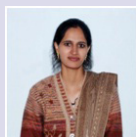
PICTORIAL ABSTRACT



SUMMARY

- *Euphorbia thymifolia* Linn whole herb methanol extract was processed through column and preparative thin layer chromatography.
- Flavan-3-ol isomers (compound I and II) has been isolated and identified.
- The compound (I) was found as catechin (2,3-trans-3,4-transflavan-3-ol).
- The compound (II) was found as epicatechin (2,3-cis-3,4-transflavan-3-ol).

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