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Research Article

Isolation and Characterization of Chemical Constituentss from *Trigonella foenum-graecum* Seed Extract

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ABSTRACT

6 new compounds were isolated for the first time from *Trigonella foenum-graecum* seed extract along with one known compound by using simple column chromatographic techniques. They were characterized by chemical and physicochemical evidence and identified as Quercetin 3-O- rutinoside, Chrysoeriol, Monotropein, Di butyl phthalate, De acetyl asperulosidic acid, 1,2 benzene di carboxylic acid and Trigoneoside II a.

INTRODUCTION

Trigonella foenum-graecum L which belongs to the Family Leguminosae has been widely cultivated in India, China, and Mediterranean countries. The seeds, leaves of this plant have been used not only as a spice or favorite food but also for various medicinal uses like antipyretic, laxative and muscle strengthening agent[4] . Isolation and characterization of some active constituents from this plant was already reported, of which furostanol steroid saponins was very difficult. These were known to have some haemolytic and adjuvant activity[4]. In the present research work trigoneoside iia was isolated using simple column chromatographic techniques. Along with this, 5 other compounds were also isolated and characterized.

MATERIAL AND METHODS

5kg of methanolic extract of *Trigonella foenum graecum* seed was taken. Its physic-chemical parameters like ash values, Extractive values, Moisture content were studied and reports were recorded. This 5kg extract was dissolved in 30 litres of water and partitioned with equal amounts of Butanol. Butanol fraction was separated and left behind water fraction was partitioned with Ethyl acetate. The three fractions butanol, ethyl acetate and water fractions were collected, distilled and dried into powder. Of all the three fractions butanol fraction was showing more prominent enrichment of the compounds in HPLC report.So butanol fraction was selected as charge material for performing column chromatography.

Simple column chromatography was used in which silica gel stationery phase was used and mobile phase was passed from highly polar to non polar solvents. Saponins were targeted in this isolation, which were polar in nature. Procedure was planned in such a way that first non polar compounds were to be separated from polar compounds from which isolation of saponins(polar) would be easier.

Column 1: 1kg of dried butanol fraction was dissolved in methanol and it was adsorbed over 1.4kg of silica gel and it was mixed to get uniform adsorption. It was dried in room temperature and ground into fine powder with out having pellets. Column was packed with silica gel and adsorbed butanol fraction was charged to that. Mobile phase was passed from 100% petroleum ether to 100% water and all the fractions were collected and dried with help of rotavapor instrument. TLC and HPLC was performed to all the collected fractions and it was found that 25%methanol/ethyl acetate and 50% methanol/ethyl acetate fractions were having better enrichment of targeted compounds when compared with standard saponin sample.

Column-2 : 25% methanol/ethyl acetate fraction was adsorbed over silica gel and charged in silica gel column.Chloroform : Methanol : Water in the ratio 6:1:0.1 , 5:1:0.1, 4:1:0.1, 3:1:0.1, 2:1:0.1, 1:1:0.1 and 50%methanol/water was used as mobile phase. All the fractions were collected, dried. TLC and HPLC was performed to those fractions.

Column-3: 50%methanol/ethyl acetate was adsorbed over silica gel(1:3)ratio and mobile phase was passed from 100%ethyl acetate to 100%methanol. All the fractions were collected and dried. TLC and HPLC was performed.From the reports it was found that in Chloroform : Methanol : Water (2:1:0.1) and 1:1:0.1 fractions, nonpolar compounds present in the initial charged butanol fraction was not present much.so in this polar fractions it was assumed to have our targeted saponins.

Column-4: Fration obtained with Chloroform : Methanol : Water (2:1:0.1) was adsorbed over Diaion NKA-9 resin and charged into diaion column. column was run in isocratic manner with 100%water only and 1litre was collected in each and every fraction, concentrated and dried.

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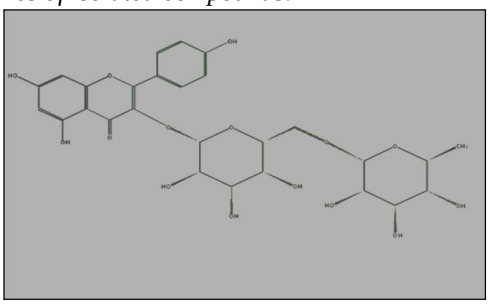
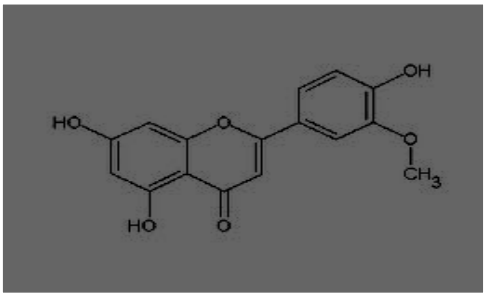
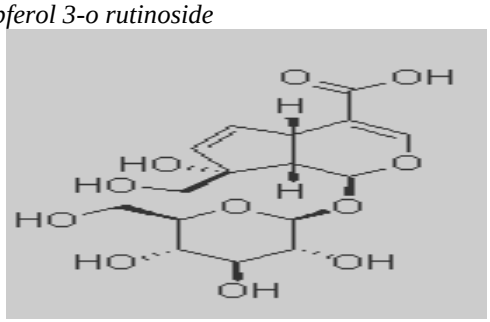
Structures of isolated compounds:

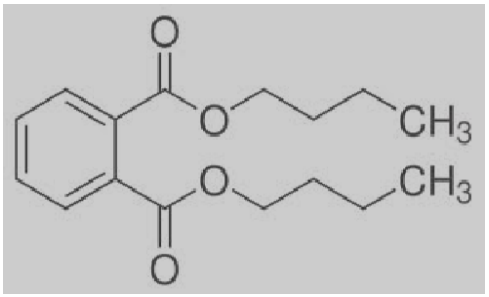




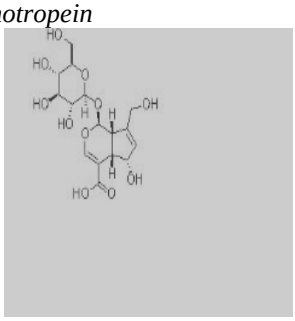
Kaempferol 3-o rutinoside



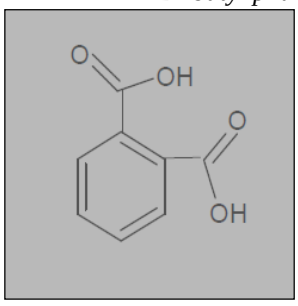
Chrysoeriol

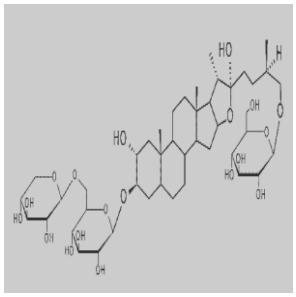


Monotropein



Di butyl phalate





De acetyl asperulosidic acid *1,2 benzene dicarboxylic acid* *Trigoneoside ii a*

Column-5: Fration obtained with Chloroform : Methanol : Water (1:1:0.1) was adsorbed over silica and charge in a silica column. Chloroform : Methanol : Water (12.5:7:1.5) was used as mobile phase and it was run in isocratic manner.20 fractions were collected in which 100ml was collected in each fraction.

Isolation of compound 1: 15% MeOH/E.A fraction from column 3 was showing only 2 spots on TLC. This fraction quantity was very less and we can't charge it to the column to separate them. Preparative TLC was performed, but as these compounds were not U.V sensitive at 254nm and 366nm identification became difficult.

Preparative TLC isolation was failed.

This 15% MeOH/E.A was dissolved in n-butanol and sonicated for 5 minutes. Even after sonication, some part of the fraction was not dissolved. Dissolved part is decanted and separated. They were dried and TLC was performed to those dissolved and undissolved portions along with initial 15%MeOH/E.A fraction. The undissolved and dissolved parts were shown single spots on TLC. They were named as TR01 and TR02.

TR01- Kaempferol-3-O-rutinoside

Molecular weight: 594;

EIMS (m/z) 594(M^+)560, 482,431,339,221,154,113(base peak)

IR (KBr) 1650.71 cm^{-1} (chelated C=O str) 3300.9 cm^{-1} (br bonded O-H str), 1178.51 cm^{-1} (C- O str), 1261.45 cm^{-1} [Ar -O–C str (aralkyl ether)],1020.34 cm^{-1} [R – O – Ar(alkyl aryl ether)], 1103.2 cm^{-1}(Ar – O – Ar str) , 2960.73 cm^{-1} (aromatic C = C str)

1HNMR:(DMSO d6) δ 2.513 (DMSO d6), δ 7.80 (s, 6H, C6 – H, C8 – H, C2'-H, C3'-H, C5'-H, C6'-H)

δ 13.20 (s, 3H, C5 – OH, C7-OH, C4'-OH) δ 3.364(s, 1H,C1''-H)

δ 0.8 to δ 1.8 (s, -CH and –OH protons of sugars)

TR02: Chrysoreol

Molecular weight:300

EIMS (m/z): 300 (M^+), 546.1, 502.1, 432.9, 391.1, 225.1, 149, 590.1(100%)

I.R (KBr)

1610.56 cm^{-1} (chelated C=O str),3259.70 cm^{-1} (br bonded O-H str),

1261.45 cm^{-1} [Ar -O–C str (aralkyl ether)],1095.57 cm^{-1} [R – O – Ar(alkyl aryl ether)],

1103.2 cm^{-1}(Ar – O – Ar str) , 2906.73 $cm^{-1,}$ 2962.66 cm^{-1} (aromatic C = C str)

^{1}HNMR: (DMSO d_6)

δ 2.512 (DMSO d_6),δ 6.80, 6.9 (s, 6H, C_6 – H, C_8 – H, $C_{2'}$-H, $C_{3'}$-H, $C_{5'}$-H,

$C_{6'}$-H), δ 13.20 (s, 3H, C_5 – OH, C_7-OH, $C_{4'}$-OH), δ 3.380(s, 1H,$C_{1''}$-H)

Isolation of TR03, TR04 FROM 2:1:0.1 fraction of column-2: Similarly 2:1:0.1 fraction from column-2 was taken and dissolved in n-butanol. Some part of the fraction left undissolved even after sonication for 10 minutes. The dissolved and un- dissolved parts were separated and dried. TLC was performed to those undissolved and dissolved parts along with initial 2:1:0.1 fraction. Dissolved and un-dissolved parts shown single spots on TLC. They were named as TR03 AND TR04 respectively.

TR03:

Molecular weight: 390

EIMS (m/z) 390 (M^+),413.1,443 455, 487, 504, 525, 541, 555,

^{1}HNMR: (DMSO d_6): δ 2.512 (DMSO d_6),δ 6.30, 6.5 (s, 6H, C_6 – H, C_8 – H, $C_{2'}$-H, $C_{3'}$-H, $C_{5'}$-H, $C_{6'}$-H),δ 13.20, δ13.5 (s, 3H, C_5 – OH, C_7-OH, $C_{4'}$-OH),δ 3.380(s, 1H,$C_{1''}$-H)

TR04: Di butyl pthalate

Molecular weight 278

278 (M^+), 279.1(M+H),301.1(M+Na), 316.1, 349.1, 363.1, 413.1, 437.1

I.R(KBr): 1660.71 cm^{-1} (chelated C=O str),3302.13 cm^{-1} (br bonded O-H str),1261.45 cm^{-1} [Ar -O–C str (aralkyl ether)],1105.21 cm^{-1} [R – O – Ar(alkyl aryl ether)],1103.2 cm^{-1}(Ar – O – Ar str) , 2924.09 cm^{-1} (aromatic C = C str)

Isolation of TR05: In column 1 85%MeOH/E.A fraction was taken and similar procedure was followed. It was dissolved in n-butanol and undissolved portion after sonication was separated and dried. TLC was performed to those dissolved and un dissolved portions. Un dissolved part gave single spot on TLC while dissolved part gave 2-3 spots. This undissolved single spot part was named as TR05.

TR05: De acetyl asperulosidic acid

Molecular weight : 390

EIMS (m/z) 390 (M^+),391.1 (M+H), 455.1, 579, 680, 764, 909, 1135.7

I.R(KBr):1610.56 cm^{-1} (chelated C=O str),3259.70 cm^{-1} (br bonded O-H str),

1261.45 cm^{-1} [Ar -O–C str (aralkyl ether)],1095.57 cm^{-1} [R – O – Ar(alkyl aryl ether)]

Isolation of TR06: In Column-4, fraction 12 was taken and dissolved in butanol. It was sonicated and dissolved and undissolved parts were collected and concentrated on water bath. Dissolved part gave single spot on TLC. It was named as TR06**.**

TR06: 1,2 benzene di carboxylic acid.

EIMS (m/z)390 (M^+),391.1 (M+H), 413(M+Na), 455, 481.

^{1}HNMR: (DMSO d_6) δ 2.512 (DMSO d_6),δ 1.2, δ 0.8 (aliphatic protons),δ 3.380(s, 1H,$C_{1''}$-H)

IR (KBr):1597.06 cm^{-1} (chelated C=O str),3147.83 cm^{-1} (br bonded O-H str)1012.63 cm^{-1} [R – O – Ar(alkyl aryl ether)] 1101.35 cm^{-1}(Ar – O – Ar str) 2924.09 $cm^{-1,}$ (aromatic C = C str)

Isolation of TR07: Ethyl acetate fraction was charged into silica column and eluted with 100% Chloroform. Here, in this column, 7 fractions were collected. TLC was performed to all fractions. In that fraction 7 was showing 2 spots. It was mixed with acetic acid and water and separated in separating funnel. Water and chloroform layers were collected separately and concentrated on water bath. Water layer showed yellow crystals after recrystallization. It gave single spot on TLC. It was named as TR07.

TR07: Trigoneoside IIa

Molecular weight: 910

EIMS (m/z):910(M^+),911 (M+H), 973 (M+64), 1052, 1148.6

IR (KBr)1664.57 cm^{-1} (chelated C=O str),3294.42 cm^{-1} (br bonded O-H str)1089.78 cm^{-1} [R – O – Ar(alkyl aryl ether)],1031.92 cm^{-1}(Ar – O – Ar str), 2924.09 $cm^{-1,}$ 2856.58 cm^{-1} (aromatic C = C str)

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