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Isolation of Flavonol of *Tephrosia purpurea*

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ABSTRACT

Flavonoids have been reported as naturally occurring compounds and considered as secondary metabolites responsible for biological activities. The flavonoids are divided into several classes, i.e., anthocyanins, flavonols, flavones, flavanones, dihydro flavonols, chalcones etc. A flavonol, Kaempferol 7-O-(rhamnosyl)-glucoside, was isolated from whole plant of *Tephrosia purpurea*. The isolated compound was identified by melting point, chemical test, IR, NMR and mass spectra.

Keywords: *Tephrosia purpurea*, Flavonol, Kaempferol 7-O-(rhamnosyl)-glucoside

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INTRODUCTION

Tephrosia Pers. (Galegeae, Lotoideae, Leguminosae) is a large tropical and sub-tropical genus of some 300 species[l]. Earlier phytochemical screening of a number of species has revealed the presence of rotenoids, isoflavones, flavanones, chalcones, flavonols and flavones [2]. *Tephrosia purpurea* Pers. occurs throughout the Indian subcontinent. This species has been reported to contain a number of rotenoids [3] besides pongamol [4], isolonchocarpin[5], karanjin, lanceolatin B, kanjone and sitosterol[6]. We now report isolation of flavonol from whole plant of *Tephrosia purpurea*.

MATERIALS AND METHODS

Plant material

The whole plant of *Tephrosia purpurea* was collected from Mandya district, Karnataka. Plant material was identified and authenticated by Dr. Raj Singh Saini, Head of the department of biotechnology, IIMT College of Medical Sciences, Meerut. A specimen number IIMT/SL/2011/11 is kept in herbarium for further reference.

Preparation of the extracts

Successive extraction

The accurately weighed 250 gm of dried powder of drug (*Tephrosia purpurea*) in three successive batches was packed in thimble flask and 750 ml of ethanol was added in 1 litre round bottom flask. The Soxhlet assembly was set up to complete 15 cycles. The same procedure repeated for two more times to get sufficient amount of extract. After that the extract was filtered and distilled under reduced pressure. The obtained extract was kept in a desiccator over calcium chloride for 3 days.

Fractionization

60 gm of *Tephrosia purpurea* was suspended in 100 ml of distilled water. This suspension was transferred to the separating funnel, to this suspension added the 100 ml of petroleum ether per batch was added into three successive times and shake for 15 min in separating funnel. The petroleum ether fraction was separated and washed with 3 ml of water, washings were transferred to the aqueous fraction. To this aqueous fraction again 100 ml of chloroform per batch was added into three successive times and shake for 15 min in separating funnel. The chloroform fraction was separated and washed with 3 ml of water; washings were transferred to the aqueous fraction. To this aqueous fraction again added the 100 ml of ethyl acetate per batch was added into three successive times and shake for 15 min in separating funnel. The ethyl acetate fraction was separated and washed with 3 ml of water; washings were transferred to the aqueous fraction. The obtained fractions (Petroleum ether, chloroform, ethyl acetate, aqueous) were distilled under reduced pressure and kept in a desiccator over calcium

chloride to obtain crude dry extracts. The obtained extracts were subjected to preliminary phytochemical analysis.

Preliminary phytochemical analysis.

Tests for Steroids and Triterpenoids

a. Libermann-Burchard test:

Extract solution mixed with few drops of acetic anhydride, boil and cool, concentrated sulphuric acid is added from the side of the test tube, A brown ring at the junction of two layers and the upper layer turns green indicates the presence of sterols and formation of deep red colour indicates the presence of triterpenoids.

b. Salkowski's test:

Dissolve the extract in chloroform with few drops of concentrated Sulfuric acid, shake well and allow to stand for some time, red colour appears in the lower layer indicates the presence of sterols and formation of yellow coloured lower layer indicating the presence of triterpenoids.

Tests for Alkaloids

a. Mayer's test: (Potassium mercuric iodide solution).

To the extract solution, add few drops of Mayer's reagent, creamy white precipitate is produced.

b. Dragendroff's test: (Potassium bismuth iodide solution).

To the extract solution, add few drops of Dragendroff's reagent, reddish brown precipitate is produced.

c. Wagner's test: (Solution of iodine in Potassium iodide).

To the extract solution, add few drops of Wagner's reagent, reddish brown precipitate is produced.

d. Hager's Test: (Saturated solution of Picric acid)

To the extract solution, add few drops of Hager's reagent, yellow precipitate is produced.

F. Tests for Phenolic Compounds

a. Ferric chloride test:

Extract solution gives blue-green colour with few drops of $FeCl_3$.

b. Shinoda Test (Magnesium Hydrochloride reduction test)

To the extract solution, add few fragments of magnesium ribbon and concentrated HCl drop wise, yellowish; yellow- orange occasionally orange colour appears after few minutes.

c. Zinc-Hydrochloride reduction test:

To the extract solution, add a mixture of zinc dust and concentrated HCl. It gives yellowish, yellow- orange occasionally orange colour appears after few minutes.

G. Tests for Flavonoids

a. Shinoda Test (Magnesium Hydrochloride reduction test)

To the extract solution add few fragments of magnesium ribbon and HCl drop wise, pink scarlet, crimson red or occasionally green to blue colour appears after few minutes.

b. Zinc-Hydrochloride reduction test:

To the extract solution, add a mixture of zinc dust and concentrated HCl. It gives red colour after few minutes.

c. Alkaline reagent test:

To the extract solution, add few drops of Sodium hydroxide solution, formation of an intense yellow colour that turns to colourless on addition of few drops of dilute acetic acid indicates the presence of flavonoids.

Isolation and identification

The aqueous fraction was subjected to column chromatography on silica gel using solvents of increasing polarities starting from chloroform, chloroform: ethyl acetate, chloroform: ethyl acetate in different ratios to yield several sub fractions. The following solvent and mixture of solvents in ml used for further elution of phytoconstituent by column-chloroform, chloroform: ethyl acetate (9:1), chloroform: ethyl acetate (8:2), chloroform: ethyl acetate (7:3), chloroform: ethyl acetate (6:4), chloroform: ethyl acetate (5:5), chloroform: ethyl acetate (4:6) chloroform: methanol (9:1), chloroform: methanol (8:2). Kaempferol 7-O-(rhamnosyl)-glucoside (1) obtained from chloroform: methanol (8:2) from aqueous fraction. It appeared as white crystalline solid (38 mg). The solvent system Butanol: acetic acid: water in the ratio 4: 1: 5 showed an Rf value 0.72.

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RESULTS AND DISCUSSION

Literature survey has revealed that plant metabolites like phenolic compounds like (simple phenols, phenolic acids, Flavonoids, tannins), and sterols play an important role in many of the activities like wound healing, analgesic, anti inflammatory ,anti oxidant and anti microbial. The structure and the presence of free hydroxyl groups in the various phenolic compounds make them an important class of compounds. The chemical activities of these phenolic compounds in terms of their reducing properties as electron or hydrogen donating is important for their anti oxidant activity [7, 8, 9]

The structures of the isolated compound was established by chemical test, melting point, IR, UV, NMR, Mass spectroscopy. The isolated compound gave a bluish green colour with ferric chloride suggesting that they are phenolic compounds. Kaempferol 7-O-(rhamnosyl)-glucoside was positive for the magnesium ribbon test suggesting it to be flavonoids.

Kaempferol 7-O-(rhamnosyl)-glucoside (1) was obtained as white crystalline solid with a melting point 180 °C. Analysis by mass spectroscopy gave molecular mass 488.48 m/z. IR (Kbr cm^{-1})- 1595 cm^{-1} (C=C), 1653 cm^{-1} (C=O), 2927 cm^{-1} (C-H stretch), 3421 cm^{-1} (OH stretch). ^{1}H NMR: Aglycone moiety- 8.32 (d, J = 9 H-$2^{1}$and H-6^{1}); 7.55 (d, J = 9 Hz, H-3^{1} and H-5^{1}); 6.21 (d, J = 2 Hz, H-6); 6.41 (d, J = 2 Hz, H-8). Sugar moiety- 5.10 (d, J = 7.5 Hz, H-1^{11}). ^{13} C: Aglycone moiety- 144.79 (C-2), 121.76 (C-3) 177.64 (C-4), 161.18 (C-5), 98.89 (C-6), 164.31 (C-7), 93.88 (C-8), 156.32 (C-9), 104.19 (C-10), 121.58 (C-1^{1}), 144.79 (C-2^{1}), 115.19 (C-3^{1}), 161.18 (C-4^{1}), 115.19 (C-5^{1}), 115. (C-6^{1}). Sugar moiety -93.63 (C-1^{11}), 71.91 (C-2^{11}), 75.92 (C-3^{11}), 68.18 (C-4^{11}), 79.20 (C-5^{11}); 68.18 (C-6^{11}).

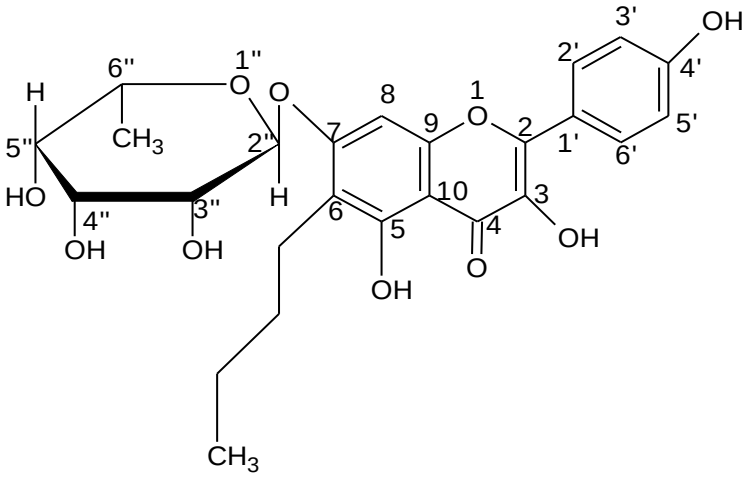

Fig.1: Kaempferol 7-*O*-(rhamnosyl)-glucoside

CONCLUSION

The current study resulted in isolation compound Kaempferol 7-O-(rhamnosyl)-glucoside (flavonol) from the aqueous fraction of ethanol extract of *Tephrosia purpurea*. The

presence of this constituent may be one of the contributing factors responsible for the activity by virtue of its different properties like anti oxidant, anti inflammatory, analgesic and antimicrobial.

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