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Research Article

**BIOGUIDED ISOLATION OF 3, 4', 5, 7
TETRAHYDROXYFLAVONE FROM *TALINUM
PORTULACIFOLIUM FORSK*****Junapudi Sunil^{1*,2}, Yasodha Krishna Janapati³, Pallaval Veera Bramhachari⁴**¹Research Scholar, Department of Pharmacy, Jawaharlal Nehru technological university, Kakinada, Andhra Pradesh, India - 533 003.²Department of Pharmaceutical Chemistry, Geethanjali College of Pharmacy, Cherryal, Keesara, Ranga Reddy District, Telangana, India- 501301.³Department of Pharmaceutical Chemistry, CHS, Mekelle University, Mekelle, Ethiopia⁴Department of Biotechnology, Krishna University, Machilipatnam, Krishna District, Andhra Pradesh-521001.**Abstract:**

Talinum portulacifolium forsk is a traditional indian medicinal, which is proved to possess a lot of biological activities. The objective of this study is to investigate of a bioassay-guided fractionation and its active components/compounds. Compounds were isolated by High performance thin layer chromatography (HPTLC) and preparative high performance liquid chromatography (pre-HPLC) and their structures were established by mass spectrometry (MS), Nuclear magnetic resonance (NMR) and Fourier transform infrared spectroscopy (FT-IR) spectroscopic analyses. One compound were isolated from Fraction-C among them, one compounds; 3, 4', 5, 7-Tetrahydroxyflavone (Acacetin) were reported for the first time in *Talinum portulacifolium forsk*.

Keywords: *Talinum portulacifolium forsk*, High performance thin layer chromatography, Mass spectrometry, 3, 4', 5, 7-Tetrahydroxyflavone

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INTRODUCTION:

Talinum portulacifolium forsk is an annual herb belongs to the family Portulacaceae [1] which mainly occurs in India, W. Peninsula, China and Ceylon. In India it is found in Andhra Pradesh and Tamil Nadu [1]. Probably, *T. Portulacifolium forsk* has been frequently used as an alternative, astringent to the bowels, worms, itching, useful in gonorrhoea [1, 2]. These experiments documented that leaves are used for diabetes, cures ulcers, antioxidant [2-4]. Therefore, the aim of the present study was to investigate the ability of *T. Portulacifolium forsk* extracts subsequently, to isolate the possible active constituent (s) through bioassay-guided fractionation techniques and identified by spectral analysis.

MATERIALS AND METHODS:

Plant materials

The plant of *T. portulacifolium forsk* was once accrued during the month of November from the wood's territory of the Tirumala Hills, Tirupathi, Chittoor district. Andhra Pradesh (India). The plant material was taxonomically identified by Dr. K. Madhava Chetty, Department of Botany, Sri Venkateshwara University, Tirupathi, Andhra Pradesh, India and a specimen was kept in the herbarium. The plant materials were washed thoroughly to remove adhering soil and earthen matter, later on sliced into thin chips and dried in shade at room temperature and then after ground to optimal coarse powder.

Chemicals and reagents

All chemicals and reagents used for CH₃OH were from ChangshuYangyuan Chemicals, China. Chloroform (CHCl₃) and EtOAc Standard Reagents, Hyderabad All of the chemicals and reagents were of analytical grade.

General experiment procedure

Infrared spectrum was obtained using Bruker Optics (Germany). ¹H NMR (400 MHz) and ¹³C NMR (100 MHz) spectra were recorded on a Bruker Avance 400 spectrometer, with CDCl₃ as a solvent and tetramethylsilane (TMS) as internal standard. NMR experiments were performed in the same spectrometer. Thin layer chromatography (TLC) was performed on 0.20 mm pre-coated silica gel aluminum sheets (Merck Kieselgel 60F254). Spots were visualized with UV light (254 nm) and sprayed with vanillin sulphuric acid. Gravity column chromatography (CC) was carried out by using Merck silica gel 60(70–230 mesh). HPTLC performed on 5X10 cm plates coated with 0.25 mm layer of silica gel 60F₂₅₄ (Merck, Darmstadt, Germany). Samples were applied as 4 mm wide bands and 6 mm apart by using a Camag (MuttENZ,

Switzerland). HPLC Shimadzu LC10 AD (Shimadzu corporation, Kyoto, Japan)

Preparation of *T. portulacifolium forsk* extract, isolation and characterization of the active constituent

The collected herbs of *T. portulacifolium forsk* were shade dried and powdered. Plant powder was then extracted three times at ambient temperature (50–60 °C), with 90% CH₃OH. During the extraction process the solvent was changed for every 24 h [5]. The CH₃OH from the pooled extracts was then removed by distillation under reduced pressure at 50–60 °C for creating extracts of *T. portulacifolium forsk* (AETP). The extracts were subjected to preliminary phytochemical investigation [6] and also for biological activity screening tests such as antioxidant activity.

The 90% of CH₃OH extracts were chosen for further isolation. The extracted solutions were suspended in distilled water and then sequentially extracted three times (2000 mL × 3) with CHCl₃ and EtOAc, CH₃OH and H₂O extracts respectively [7]. All these fractions were screened for antioxidant activity. The potential active fractions were submitted on a silica gel column for further fractionation. These fractions were collected and combined after HPTLC analysis for yielding different fractions. The fractions possessing the potential pharmacological activity were consecutively re-chromatographed on a silica gel column. These fractions were further followed by semi-preparative HPLC using 55% methanol solution as mobile phase to obtain the final purified compounds.

The purity of isolated compound was established by structural analysis and was confirmed by the interpretation of the spectral data (FT-IR, ¹H, ¹³C NMR and MS) and further tested for its pharmacological activities.

Determination of Total phenolic content

An aliquot of 100 μL of extract was mixed with 2.5 mL of Folin-Ciocalteu phenol reagent and allowed to react for 5 min. Then, 2.5 mL of saturated Na₂CO₃ solution were added and allowed to stand for 1 h before the absorbance of the reaction mixture was read at A₇₂₅ nm [8]. The phenolic compound substance was resolved as phenolic compound content was determined as GAE using the linear equation based on the calibration curve: $C = (c \times V/m)$ Where, C = total content of phenolic compounds (mg/g plant extract in GAE); c = concentration of gallic acid obtained from calibration curve (mg/mL); V = the volume of the sample solution (mL); m = weight of the sample (g) [9]. All tests were conducted in triplicate.

Determination of total flavonoid content

TFC of the plant crude extracts was determined spectrophotometrically using the Ghasemzadeh method [10] with little modification in the protocol. The test sample (1 mL) was placed in a 10-mL volumetric flask Mixed with Ethanol (60%, 8 mL) added after 6 min AlCl_3 (10%, 0.2 mL), NaOH (4%, 0.6 mL) was added and then water to a volume of 10 mL. Read the absorbance of the reaction mixtures at A_{430} nm (Spectrophotometer). A standard curve was plotted using quercetin as a standard 20-250 mg/mL. The TFC of the extracts was expressed as mg quercetin equivalents per gram of plant material on dry weight basis[10]: $H = (c \times V/m)$ Where, H = total content of flavonoid compounds (mg/g plant extract in GAE); c = concentration of quercetin obtained from calibration curve (mg/mL) V = the volume of the sample solution (mL); m = weight of the sample (g); All tests were conducted in triplicate.

RESULTS:

Extraction, Fractionation, and Isolation of Antioxidant Compound:

The *T. portulacifolium forsk* herbs were shade dried and powdered. *T. portulacifolium forsk* (600g) plants powders was extracted three times at ambient temperature (50-60°C) with 90% CH_3OH . During the extraction with solvents, the solvent was changed by every 24h. The CH_3OH from the pooled extracts was removed by distillation under reduced pressure at 50-60°C to create AETP (80.2g). The extracts were subjected to preliminary phytochemical investigation and were further subjected for the biological activity

screening tests. Therefore, the 90% CH_3OH extract (AETP) was chosen for the following isolation.

The extracted solutions (AETP 60.2g) were suspended in distilled water and then sequentially extracted three times (2000mL \times 3) with CHCl_3 and EtOAc, CH_3OH and H_2O extracts, produce CHCl_3 (5.6g), EtOAc (22.1g), CH_3OH (6.8g) and H_2O (8.6g) extracts respectively. Among these fractions the EtOAc-soluble fraction was found to have the potential antioxidant activity. Therefore the EtOAc-soluble fraction (20 g) was submitted on a silica gel column (10 \times 100cm) using a gradient of ether-EtOAc (5L) 100:0, 96:4, 92:8, 90:10, 80:20 and 70:30. Fractions of 5L were collected and combined after TLC analysis to yield 7 fractions of each plant (A – G). *T. portulacifolium forsk* Fraction C possessed the highest antioxidant was consecutively re-chromatographed on a silica gel column (5 \times 70cm, 260g) using a gradient of EtOAc (2L) 100:0,80:20,60:40,40:60,20:80. Fractions of 100 mL were collected and combined after TLC analysis to yield fractions of *T. portulacifolium forsk*. The extract [fraction-A(2.45g)] the extraction and isolation procedure of *T. portulacifolium forsk* is shown in figure-1, HPTLC fingerprints of Solvent and fra compound-A (Figure-2)

These fractions were followed by semi-preparative HPLC using 55% methanol solution as the mobile phase to obtain compound-A (32 mg) of *T. portulacifolium forsk* (figure-3), as a result, a known 3, 4', 5, 7-Tetrahydroxyflavone agrees in every respect (IR, NMR and MS) with those of the results table-1

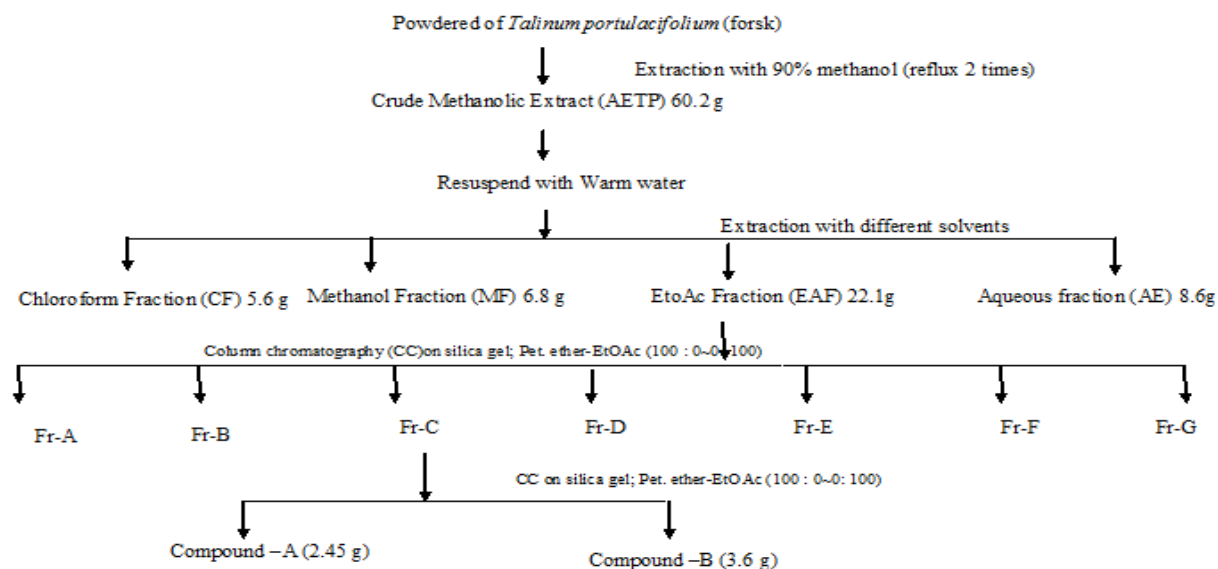
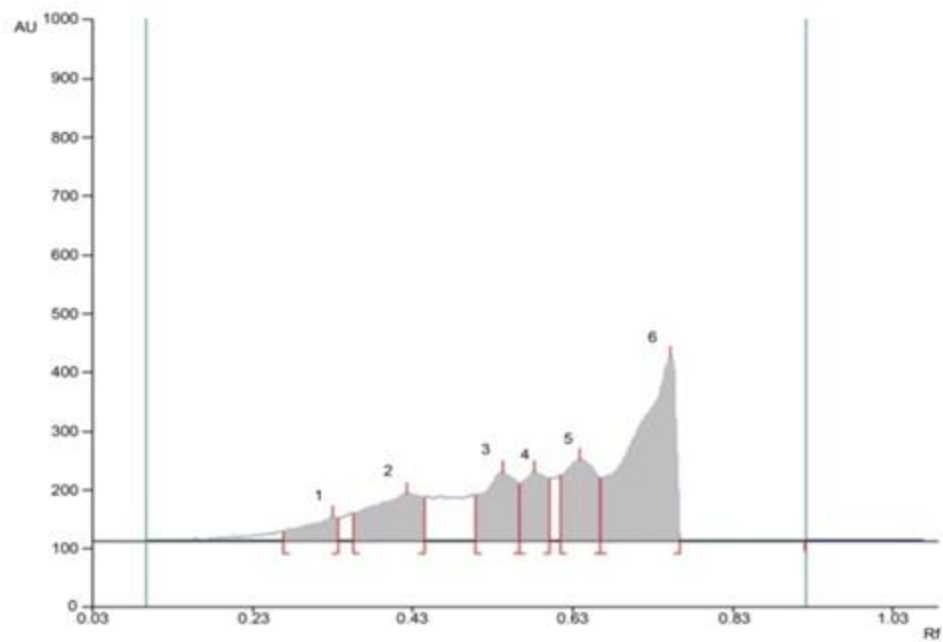
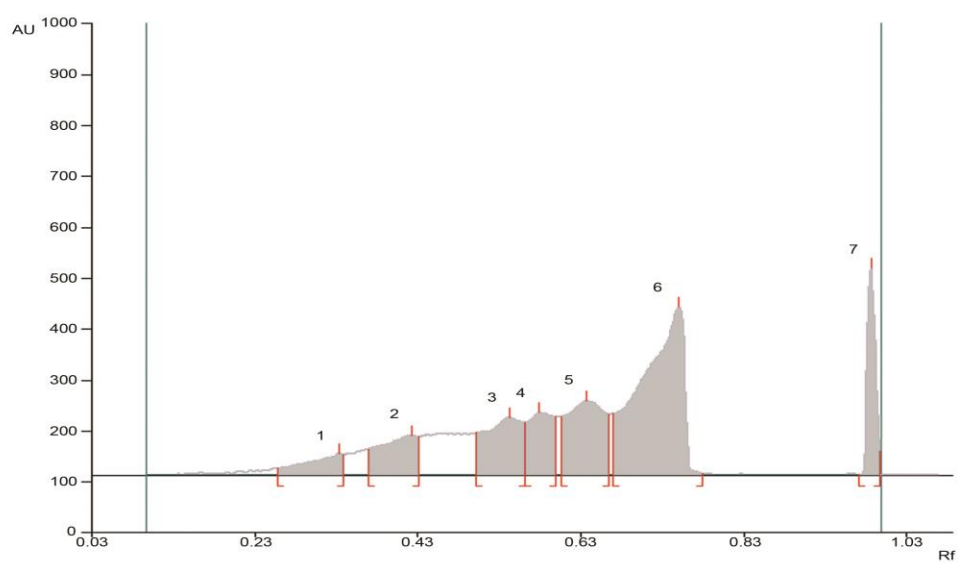


Fig1: Extraction scheme for the isolation of each compound from *Talinum portulacifolium forsk*

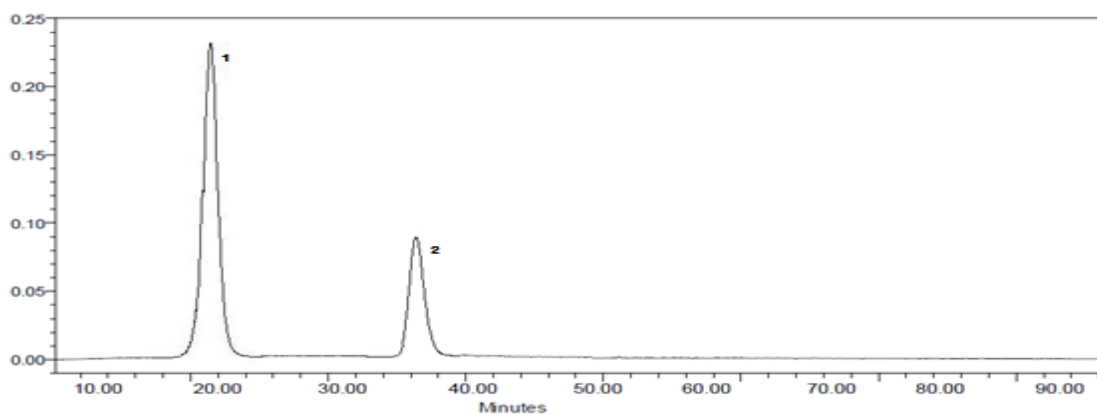


(A)

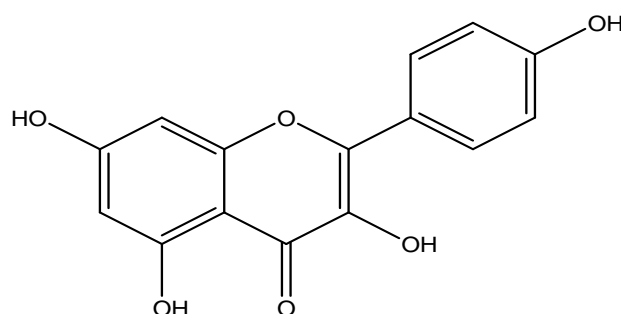


(B)

Fig. 2: HPTLC fingerprints of Solvent (A) and TPF-1 (B)



(C)



(D)

Fig. 3 HPLC fingerprints of Compound-A of EAF extracts from *Talinum portulacifolium forsk* chemical structures of the compounds (C and D).

Table 1: Tables showing the chemical constituents of the active Compound-A respectively, as elucidated by FT-IR,¹H NMR, MASS Preliminary Phytochemical Studies

| Compound | Solvent system | Rf Values at 254 nm | IR spectra | ¹ H NMR spectra | Mass spectrum |
|----------|---|---------------------|--|--|--|
| TPF-1 | Ethyl acetate:n-Butanol:n-Propanol: Methanol: water (60: 20:10:5:5) | 0.93 | OH-stretch-3217, C=O arylketone 1650; C=C stretch 1620, C-Cstretch 1503,1573; C-O-C 1209; C-H stretch 2817,2657. | δ 7.4, 6.9 (4H, d,j 8.2,1.0), δ 6.4 (2H, s), δ 12.4,10.3,5.9 (4OH, s) | 286 (M.F. C ₁₅ H ₁₀ O ₆) m/z: 270,260,234,194. |

The extract of *T. portulacifolium forsk* were subjected to preliminary phytochemical screening for the detection of various phytochemical constituents such as alkaloids, amino acids, carbohydrates, flavonoids, glycosides, mucilage, proteins, steroids, tannins and terpenoids.

Determination of Total flavonoid content and Total phenolic content.

The total phenolic contains amount was calculated as quite high in alcoholic extraction of *T. portulacifolium forsk* 35.62±8.5 µg /mg GAE /100 g respectively.

The total flavonoid contents in the examination of plant extracts are expressed in term of quercetin equivalent respectively. The values obtained for the con.c of TPC were measure in *T. portulacifolium forsk* as 194.89 ± 2.23g quercetin/100g. The AETP may be responsible for its high free radical scavenging activity due to presence of hydroxyl groups in the polyphenolics and flavonoids.

DISCUSSION:

Flavonoids, a major group of polyphenols, are considered to be the active principles in diverse medicinal plants and have been reported to possess numerous pharmacological properties [11, 12]. The most essential biological activity of flavonoids is mainly due to their antioxidant property by acting as radical scavengers [13], hydrogen donors, reducing agents and peroxidation inhibitors [14, 15]. Previous studies reported that the pharmacological effects of *T. portulacifolium forsk* such as Antidiabetic activity, Antipyretic Activity, Antibacterial activity, were recognized due to the presence of the phenol and flavonoid compounds. In the present study, the isolated compound was identified as flavone compounds

The results of the present work also propose that the numerous pharmacological properties exerted by *T. portulacifolium forsk* may be partly due to the presence of antioxidant flavone compound.

CONCLUSION:

Taking into account our present study, it can be concluded that the ethyl acetate extract of *T. portulacifolium forsk* and its main flavonoid, 3, 4', 5, 7-Tetrahydroxyflavone (kaempferol). flavonoid also support the ethnobotanical use of this plant in traditional medicines of several countries including India.

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Conflict of interest

The authors declare that they have no conflicts of interest.

REFERENCES:

1. Sunil J, Nath MS, Vinatha B. Phytochemical studies on *Talinum Portulacifolium* (forsk) *Sci. J Pharm*, 2010; 1(1):1-4.
2. Rao TN, Kumarappan CT, Lakshmi MS, Mandal SC. Antidiabetic activity of leaves of *talinum portulacifolium* (forssk) in alloxan-induced diabetic rats. *Pharmacol online*, 2007; 2 : 407-417.
3. Gundamaraju R, Maheedhar K, Hwi KK. Exploiting the phenomenal anti-ulcerogenic potential of *Talinum portulacifolium* ethanolic extract whole plant on Albino Rats. *Pharmacogn Res*, 2014; 6 : 227-233.
4. Adithya ES, Sasikumar JM, Krishnakumar KA, Lakshmi MS, Christabel PH. In vitro antioxidant activity mineral content and HPLC analysis of *Talinum portulacifolium* (forssk.) Asch ex schweinf leaf and stem. *Int J Pharm Pharm Sci*, 2014; 4 (4) : 423-429.
5. Mallikarjuna, Venkateswara S, Pradesh A. In vitro antioxidant and free radical scavenging potential of *holostemma ada - kodiensis* k. Schum. *IJPSR*, 2011; 2(9):2413-2318.
6. Souza Brito ARM. How to study the pharmacology of medicinal plants in underdeveloped countries. *J Ethnopharmacol*, 1996; 54(2-3):131-138.
7. Orhan I, Küpeli E, Terzioğlu S, Yesilada E. Bioassay-guided isolation of kaempferol-3-O-β-D-galactoside with anti-inflammatory and antinociceptive activity from the aerial part of *Calluna vulgaris* L. *J Ethnopharmacol*, 2007; 114(1):32-7.
8. La Cava EL, Sgroppo SC. Evolution during refrigerated storage of bioactive compounds and quality characteristics of grapefruit [*Citrus paradisi* (Macf.)] juice treated with UV-C light. *LWT-Food Science and Technology*, 2015; 63(2):1325-33.
9. Ibrić A, Čavar S. Phenolic Compounds and Antioxidant Activity of *Cocoa* and *Chocolate* Products. *Bulletin of the Chemists and Technologists of Bosnia and Herzegovina*. 2014; 42:37-40.
10. Ghasemzadeh A, Jaafar HZE, Rahmat A. Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (*Zingiber officinale* Roscoe). *Molecules*, 2010; 15(6):4324-4333.

11. Harborne JB, Williams CA. Advances in flavonoid research since 1992. *Phytochemistry*, 2000;55(6):481–504.

Calado JC, Albertão PA, de Oliveira EA, Letra MH, Sawaya AC, Marcucci MC. Flavonoid Contents and Antioxidant Activity in Fruit, Vegetables and Other Types of Food. *Agricultural Sciences*. 2015;6(04):426.

12. Gupta P, Goel R, Agarwal AV, Asif MH, Sangwan NS, Sangwan RS, Trivedi PK. Comparative transcriptome analysis of different chemotypes elucidates withanolide biosynthesis pathway from medicinal plant *Withania somnifera*. *Scientific reports*. 2015 ;5:18611.

13. Van Acker SABE, Van Den Berg D, Tromp MNJL, Griffioen DH, Van Bennekom WP, Van Der Vijgh WJF. Structural aspects of antioxidant activity of flavonoids. *Free Radic Biol Med*, 1996;20(3):331–342.

14. Nijveldt R J, Van Nood E L S, Van Hoorn D E, Boelens P G, Van Norren , Van Leeuwen PA. Flavonoids a review of probable mechanism of action and potential applications. *Am J Clin Nutr*, 2001;74(4):418–425.

15. Tohidi B, Rahimmalek M, Arzani A. Essential oil composition, total phenolic, flavonoid contents and antioxidant activity of *Thymus* species collected from different regions of Iran. *Food Chem*, 2017;220:153–161.