RESEARCH ARTICLE

Estimation of Gallic acid, Rutin and Quercetin in *Portulaca* quadrifida L. - A potential wild edible plant by HPTLC method

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ABSTRACT

HPTLC method was developed for the quantitative estimation of gallic acid, rutin and querecetin from methanolic extract of *Portulaca quadrifida* L. a potential wild edible plant. Precoated silica gel GF₂₅₄ used as stationary phase and mobile phase for gallic acid was Toulene: Formic acid: Ethyl acetate: Methanol [3:3:8:2, V/V/V/V] and Mobile phase for rutin and querecetin was Ethyl Acetate: Formic acid: Glacial Acetic acid: Water [10:0.5:0.5:1.3, V/V/V/V]. Detection and quantification were performed densitometrically at wavelength λ 254. The Rf values of gallic acid, rutin and querecetin are 0.41, 0.19 and 0.94 respectively. The total peak areas of the standards (gallic acid, rutin and querecetin) and the corresponding peak areas of extract were compared and the gallic acid, rutin and quercetin content were estimated to be 790.9, 2029.7 and 4326.0.

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INTRODUCTION

Portulaca quadrifida L. is a small diffused, succulent annual herb found throughout the tropical part of India. It is said to be useful in asthma, cough, urinary discharges, inflammations, and ulcers, abdominal complaints (Kirtikar and Basu, 2001). It has been reported to possess antifungal activity (Hoffman et al., 2004). Plant shows anti-diabetic properties (Khatun et al., 2015). Fresh leaves of P. quadrifida slightly wormed and applied topically in joint swelling (Abbasi et al., 2013), It shows depressant effect of ethanolic extract on CNS (Syed et al., 2010). The leaves and tender shoots of plant are cooked as vegetables by tribal and local peoples in Maharashtra and rest part of India (Reddy 2012; Naik 1998; Raphel and Britto 2015).

Wild edible plants play a very important role in the diet of tribal communities. They are major source of food for tribals of forest areas. Edible parts of wild plants are promising gift of nature to mankind, these are not only delicious and refreshing but also the chief source of vitamins, minerals, proteins and other nutrients.

'Nutraceutical' the term coined in 1979 by Stephan De Felice. It is designed as a food or parts of food that provide medical or health benefits, including the prevention and treatment of disease (De Felice 1992). Nutraceutical may range from isolated nutrients, dietary supplements, herbal products and processed products. Nutraceutical play important role in physiological benefits or provide protection against the diseases (Rajsekaran *et al.*, 2008).

The major nutraceutical ingredients in plant are phenolic compounds mainly Flavonoids (Tapas *et al.,* 2008). Gallic acid (3, 4, 5,-tryhydroxybenzoic acid) is naturally occurring polyphenolic compounds posses astringent, antioxidative, antimicrobial activity (Prince *et al.,* 2009, Urizzi *et al.,* 1999, Verma *et al.,* 2013). They also constitute an unavoidable component of the diet. Rutin and quercetin are phenolic compounds exhibit antiulcer, anti-inflammatory, antioxidant, antimicrobial, antiallergic activity (Agnes *et al.,* 2008, Maalik *et al.,* 2014, Gupta *et al.,* 2014, Singh and Bilashini 2015). They have shown regulatory activity of hormones such as transport, metabolism and action of thyroid harmones (Ashok *et al.,*2010).

High performance thin layer chromatography (HPTLC) has emerged as an useful analytical method for qualitative and quantitative estimation of chemical constituents present in plant materials (Sethi, 1996). Present study deals with estimation of important nutraceuticals and antioxidants like gallic acid, quercetin and rutin in *Portulaca quadrifida* L. by HPTLC method.

MATERIAL AND METHODS

Preparation of extract

The aerial part of wild edible plant *Portulaca quadrifida* L. were collected from different parts of Nanded district. The plant was identified and authenticated. Edible part of plants were dried and made into coarse powder and stored in sealed container. Powder then extracted with methanol by Soxhlet apparatus and concentrated.

Reagents and other materials

Gallic acid, rutin and quercetin [Sigma Aldrich] toluene, formic acid, ethyl acetate, methanol, glacial acetic acid, [all reagents of analytical grade, E-Merck] and silica gel F₂₅₄ TLC aluminium plates [E-Merck].

Preparation of standard and sample solutions

Gallic acid, rutin and quercetin 10mg were accurately weighed into 10mL volumetric flask dissolved in 10 mL of methanol [1mg/mL]. The 100 mg of extract was dissolved in methanol [10mL] then solution was filtered through whattman filter paper No. 42.

Development of HPTLC Technique

The sample were spotted in the form of bands with micro litre syringe on pre-coated silica gel plates F_{254} [10 cm x 10 cm with 0.2 mm thickness] using CAMAG Linomat 5 applicator automatic sample spotter of band width 6mm. The plates were developed in a solvent system in CAMAG glass twin through chamber previously saturated with the solvent for 30 min. The distance was 8 cm subsequent to the scanning, TLC plates were air dried and scanning was performed on a CAMAG TLC Scanner in absorbance at 254 nm and operated with win CATS Planar chromatography Manager.

Gallic acid estimation in P. qudrifida L.

Stationary phase- silica gel F_{254} plates, Mobile phase-Toulene: Ethyl acetate: Formic acid: Methanol [3:3:8:2 v/v/v/v/v], standard Gallic acid 1 mg/ml[5 μ l], sample Methanol extract 10mg/ml[10 μ l], Migration distance 80 mm, scanning wavelength 254 mm, Mode of scanning Absorption [deuterium].

Rutin and Quercetin estimation in P. quadrifida L.

Stationary phase- silica gel F_{254} plates, Mobile phase-Ethyl acetate: Formic acid: Glacial acetic acid: Water [10:0.5:0.5:1.3 v/v/v/v/v], standard Rutin and Quercetin 1 mg/ml [5 μ l], sample Methanol extract 10mg/ml [10 μ l], Migration distance 80 mm, scanning wavelength 254 mm, Mode of scanning Absorption [deuterium].

RESULTS AND DISCUSSION

The Rf value of standard gallic acid was found to be 0.39 and the peak area 18780.5 [fig. 1]. Methanolic extract of *P. quadrifida* L. showed seven peaks [fig.2], the third peak Rf value 0.41was coinciding with standard Rf values and its peak area was 790.9. The Rf values of standard rutin and quercetin was found to be 0.14 and 0.92 and peak area was 16522.0 and 8088.1 respectively [fig.3, 4]. Methanolic extract of plant showed eight peaks the third and seventh peaks of Rf values 0.19 and 0.94 was coinciding with standard Rf value, peak area was found 2029.7 for rutin and 4326.0 for quercetin [fig.5].

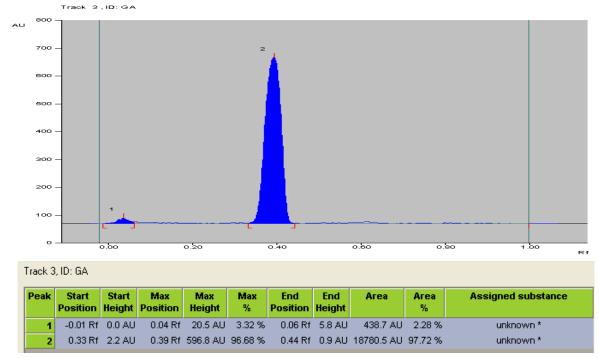


Figure 1: HPTLC Profile for Gallic acid standard

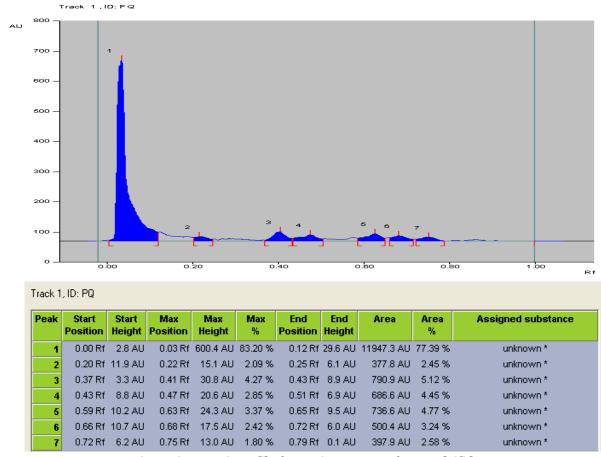


Figure 2: HPTLC Profile for MeOH Extract of P. quadrifida L.

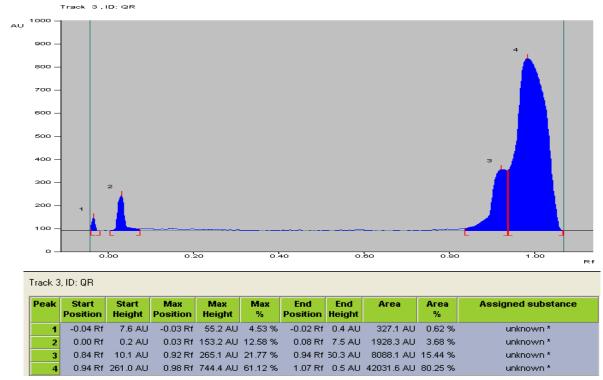


Figure 3: HPTLC Profile for Quercetin standard

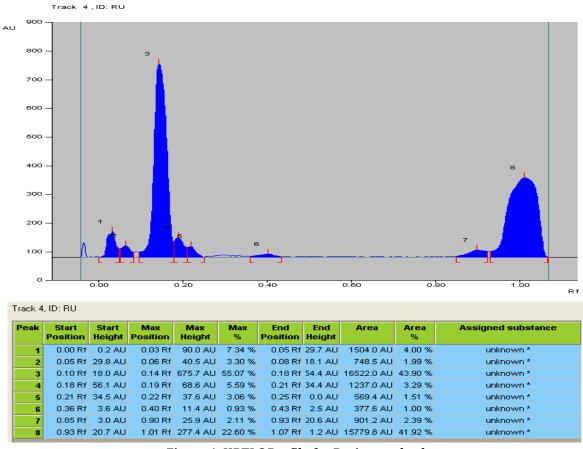


Figure 4: HPTLC Profile for Rutin standard

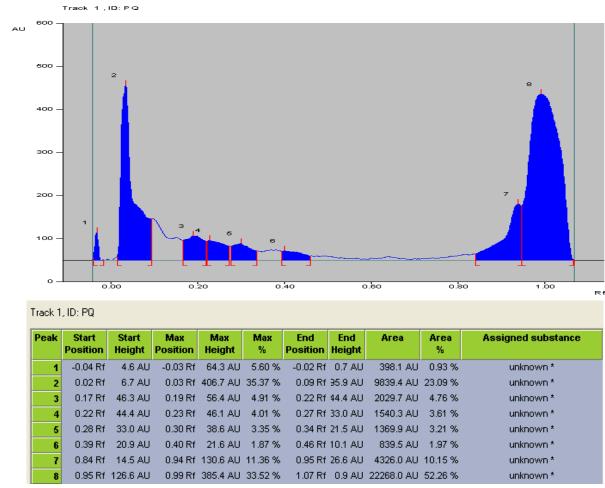


Figure 5: HPTLC Profile for MeOH Extract of P. quadrifida L.

CONCLUSION

HPTLC Analysis of *P. quadrifida* shows good concentration of gallic acid, quercetin and rutin which proves its antioxidant nature. The results of present study support its edible nature and it could be potential source of nutraceutical and natural antioxidant.

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