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**RESEARCH ARTICLE**

# CHROMATOGRAPHY FINGER PRINT PROFILING AND PHYTOCHEMICAL INVESTIGATION ON LEAF AND BARK METHANOLIC EXTRACT OF *OUGEINIA OOJEINENSIS* (ROXB.) HOCHR.

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**ABSTRACT**

Greenbelts *Ougeinia oojeneinensis* (Roxb.) Hochr. is a very important medicinal plant in the deciduous forest. Whole parts of the plant are rich in secondary metabolite, which impart miraculous medicinal uses to the plants. Present investigation was designed to evaluate the preliminary phytochemical test on leaf and bark methanolic extract of *Ougeniao oojeinensis*. The results of the preliminary phytochemical studies confirms the presence of carbohydrate, saponin, starch, flavonoids, steroids, alkaloids, glycosides, terpenoids, and proteins in the methanolic extracts of *Ougenia oojeinensis* bark and leaves. This study also estimated the Rutin and Qurecetin present in the methanolic leaf and bark extract of the *Ougeinia oojeinensis* through HPTLC method. The solvent system used for the quantification of quercetin and rutin was Toluene: Ethyl acetate: Formic acid (6: 4: 0.8 v/v/v). The max Rf values of quercetin and rutin were 0.51 and 0.04 respectively. HPTLC fingerprint analysis of leaf and bark extract of *Ougeinia oojeinensis* can be used as a diagnostic tool for the correct identification of the plant. The present HPTLC profile is useful in differentiating the species from the adulterant and act as a biochemical marker for this medicinally important plant in the pharmaceutical industry and plant systematic studies.



## INTRODUCTION

India has the great topographic and climatic diversity because of which it shows very rich and diverse flora and fauna (**Sinhababuand Banerjee, 2013**). The uses of plants as medicines have been practiced from date back to prehistoric period. Approximately 3000 plants species are known to have medicinal properties in India (**Majumder and Paridhavi 2013**). In all over world Herbal medicines have been used as main source of primary healthcare (**Meena *et al.*, 2009**). Plants used in traditional medicine have stood up to the test of time and contributed many novel compounds for preventive and curative medicine to modern science. India is sitting on a gold mine of well recorded and traditionally well practiced knowledge of herbal medicine (**Umadevi *et al.*, 2013**). This traditional medicinal system is safer and cheaper (**George 2011**). In the modern word it has been realized the herbal drugs strengthens the body system specifically and selectively without side effects (**Basha *et al.*, 2013**). It has been briefly described that traditional herbal medicinal system has vital importance in developed countries (**Soetan and Aiyelaagbe 2009**). Tribal people gain the money in his pocket with use of herbal medicinal practices adopted by traditional medicine-man and healer in Central India for curing of various diseases (**Nath and Khatri 2010**). *Ougenia oojeinensis* (Roxb.) Hochr., Is 6-12 m high tree, with short crooked trunk, bark dark brown, deeply cracked, branches slender. It is distributed in sub Himalayan tract and outer Himalayan valleys and slopes up to 5000 ft. from Punjab to Bhutan, Chota Nagpur, Cental India, Orissa, the central Provinces, Bombay, Marwar of Rajputana. Plant bark is used for anthelmintic, astringent to the bowels, cures 'Kapha' and 'Vata', dysentery, leucoderma, urinary discharges, blood diseases, ulcers, skin diseases, biliousness, burning sensation and anaemias. Among the hill tribes of Chota Nagpur a decoction of the bark is given when urine highly coloured (**Kirtikar and Basu, 1975**). It shows potential antibacterial, antioxidant and anti-cancerous activity (**Singh *et al.*, 2011**). It is also good oral hypoglycemic agents and used for anti- depressant activity (**Velmurugan *et al.*, 2013; Sindhu and Sharma 2013; Malvi *et al.*, 2011**). It is used to treat Diabetes mellitus (**Elavarasi *et al.*, 2013**). It shows significant protective effect against hepatotoxicity induced by carbon tetrachloride (**Sahu and Roy, 2009**). Flavonoids mainly homoferreirin and ougeinin are present in the bark (**Samyal *et al.*, 2013**). The wood is used for timber, tree is lopped for fodder and it yields a bast fibre useful for cordage (**Annonymous, 1948**). The phytochemical analysis of the medicinal plants is important and has commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for treatment of various

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diseases (**Wadood *et al.*, 2013**). Among the modern analytical tools HPTLC is a powerful analytical method equally suitable for qualitative and quantitative analytical tasks (**Andola, 2010**). HPTLC offers better resolution and estimation of active constituents can be done with reasonable accuracy in a shorter time (**Syed *et al.*, 2013**).

The two flavonoid, rutin and quercetin in the extracts of leaves and bark exhibit notable pharmacological activities (**Behnaz *et al.*, 2013**). Quercetin is controlling oxidative stress and reducing the incidence of cardiovascular diseases (**Lakhanpal and Rai 2008**). It also acts as an antioxidant and anti-inflammatory agent and it has potent anticarcinogenic properties as apoptosis inducer (**Akan and Garip, 2011**). They also have reduced blood pressure and a lower risk of stroke. Quercetin may treat and preventtype 2 diabetes and decrease the incidence of Alzheimer disease (**Okasha and Bayomy 2010**). Rutin exhibits multiple pharmacological activities including antibacterial, Antioxidant, antitumour, antiinflammatory, antidiarrhoeal, antiulcer, antimutagenic, myocardial protecting, vasodilator, immunomodulator and hepatoprotective activities (**Janbaz *et al.*, 2002, Hunyadi *et al.* 2012**). It maintains healthy collagen, which keeps our skin healthy and firm. It also helps to increase capillary strength and to regulate their permeability (**Sattanathan *et al.*, 2011**). Through HPTLC method important constituent present in plant i.e. Rutin and quercetin of *Ougenia oojeinensis* is estimated which play important role in the biological activity.

## MATERIALS AND METHODS

### Collection of Plant Material

Leaves and bark of *Ougenia oojeinensis* (Roxb.) Hochr. were collected from Kinwat forest (190 51' 14.53''N 77055'08.92''E) in Nanded district of Maharashtra. Specimen were identified and authenticated by Dr. R. M. Mulani, Department of Botany, School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded. Freshly collected leaves and stem bark of *Ougenia oojeinensis* were dried in shade and pulverized to coarse powder. The powder was stored in an airtight container and kept in a cool, dark, and dry place (**Hassan *et al.*, 2014; Das *et al.*, 2014**).

### Method of preparation of methanol extract

The extraction was done by using Soxhlet apparatus. The 25 gm powder of leaves and bark were extracted using 250 ml methanol for 72 hours (**Vijayalakshmi *et al.*, 2012**). The methanolic extract of bark and leaves of *Ougenia oojeinensis* were used for this study.

### Preliminary phytochemical screening

The presence of various phytoconstituents such as steroids, alkaloids, terpenoids, glycosides, flavonoids and carbohydrates were screened in the methanolic leaf and bark extracts of *Ougenia oojeinensis* (**Harborne, 1973; Gomathi *et al.*, 2013; Santanu *et al.*, 2011; Ali 2000; Jerald and Jerald, 2007**).

### HPTLC Profile (High Performance Thin Layer Chromatography)

HPTLC studies were carried out by following the method of Harborne and Wagner *et al.* (**Harborne 1984, Wagner *et al.*, 1996**).

**Fig. 1. Showing *O. oojeinensis* plant habit**

**Fig. 2. Showing *O. oojeinensis* stem**

### Preparation of standard and stock solution

**Preparation of Standard solution of quercetin:** 10 mg accurately weighed quercetin were dissolved in methanol and made the volume up to 10 ml with methanol to get final concentration of 1000 µg/ml (**Swaroop *et al.*, 2005**).

**Preparation of Standard solution of Rutin:** 10 mg accurately weighed rutin were dissolved in methanol and making the volume up to 10 ml with methanol to get final concentration of 1000 µg/ml (**Pawar and Salunkhe, 2012**).

**Preparation of sample solution for Rutin and quercetin estimation:** 100 mg accurately weighed sample extract were dissolved in methanol and making the volume up to 10 ml with methanol to get final concentration of 10 mg/ml. The solution was filtered through membrane filter paper.

**Developing Solvent System**

A number of solvent systems were tried for the extracts but the satisfactory resolution was obtained in the Mobile phase Toluene: Ethyl acetate: Formic acid (6:4:0.8) solvent system (**Leela Saraswathy 2013**).

**Sample application**

On the precoated silica gel GF254 plates (20 cm×10 cm with 0.2 mm thickness, E.Merck) samples were spotted in the form of bands with CAMAG microlitre syringe using camaglinomat V. Automatic sample spotter of band width 7 mm. CAMAG glass twin trough chamber previously saturated with the solvent for 30 min were used for the plate development (**Arunachalam *et al.*, 2013, Deepa *et al.*, 2013, Srinivasa *et al.*, 2004**).

**Development of Chromatogram**

After the application of sample, the chromatogram was developed in Twin trough glass chamber 20 x 10 cm saturated with solvent system Toluene: Ethyl acetate: Formic acid (6:4:0.8) to methanolic extract for 15 minutes.

**Detection of Spots**

The air-dried plates were viewed in ultraviolet radiation to mid-day light .Scanning of air dried TLC plates was performed on a CAMAG TLC scanner in absorbance at 254 nm and 366 nm operated by Wincats software 4.03 versions (**Sushma *et al.*, 2013**).

**Specificity**

The specificity of the method was ascertained by analyzing standards and sample extract by simultaneously applying on the same TLC plate. The spots of rut in and quercetin in the sample extract were confirmed by comparing the Rf values and spectra of the spots with those of respective standards. The peak purity was checked by comparing the spectra at three different levels, i.e. Peak start, Peak apex and peak end (**Tambe *et al.*, 2013**)

## RESULTS AND DISCUSSION

**Preliminary phytochemical screening**

Indian system of medicine has a long history of use but they lack adequate scientific documentation, particularly in light of modern scientific knowledge. The medicinal value of plant lies in the bioactive phytochemical constituents of the plant and which shows various physiological effects on human body. So through phytochemical screening one could detect the various important compounds which could be used as the base of modern drugs for curing various diseases (**Sheikh *et al.*, 2013**). The Preliminary phytochemical screening of the plants is primarily an important aspect in finding the chemical constituents in plant materials. Hence the present study was qualitative analysis and quantitative estimation of phytoconstituents (**Eswari *et al.*, 2013**).

The plant *Ougenia oojeinensis* is important therapeutic plant in the Indian medicinal system (**Srivastava *et al.*, 2012**). A preliminary phytochemical screening is useful standard qualitative procedures to reveal the presence of several secondary metabolites in the selected medicinal plant extract (**Sathya *et al.*, 2013**). Previous study showed the presence of Saponins, Glycosides, Carbohydrates, Tannins, Phenolic compounds, Flavonoids, Gums and Mucilage in both the ethanolic and aqueous extracts while alkaloid was present in the ethanolic (**Gunasekaran *et al.*, 2010**) extract. Phytochemical analysis of the methanolic extract is yet to done. The preliminary phytochemical screening of *Ougenia oojeinensis* inmethanolic extract of leaves and bark was carried out for the detection of various phytoconstituents. The results of the preliminary phytochemical studies confirms the presence of carbohydrate, saponin, starch, flavonoids, steroids, alkaloids, glycosides, terpenoids, and proteins in the methanolic extracts of *Ougenia oojeinensis* bark and leaves. The results are shown for Screening in Table 1.

**Table 1. Qualitative analysis of phytoconstituents of leaf and bark of O. *oojeinensis***

| S.No | Test for constituent | Leaf | Bark |
|---|---|---|---|
| 1 | Steroids | | |
| | a. Lieberman- Bucharad's test | + | + |
| | b. Salkowski test | + | + |
| 2 | Alkaloids | | |
| | a. Hager test | + | + |
| | b. Wagner test | + | + |
| | c. Mayer test | + | + |
| 3 | Terpenoids | + | + |
| 4 | Glycosides | | |
| | a. Molish test | + | + |
| 5 | Flavonoids | | |
| | a. Shinoda test | + | + |
| 6 | Carbohydrates | | |
| | a. Anthrone test | + | + |
| | b. Benedict test | + | + |
| | c. Fehling test | + | + |
| | d. Molish test | + | + |
| 7 | Protein | | |
| | a. Biuret's test | + | + |
| 8 | Resin | - | - |
| 9 | Saponins | + | + |
| 10 | Tannin | + | + |
| 11 | Starch | + | + |

HPTLC is an effective and powerful method which can be used for pharmaceuticals analysis, plant constituents, and bio macro molecules. Several samples can be run simultaneously using a small quantity of mobile phase, thus lowering analysis time and cost per analysis with increasing demand for herbal products as medicines and cosmetics there is an urgent need for standardization of plant products (**Starlin and**

**Gopalakrishnan, 2013**). Methanolic extract of the bark and leaf were used for the HPTLC fingerprint of *Ougeinia oojeinensis* plant, with solvent system Toluene: Ethyl acetate: Formic acid (6:4:0.8). Air dried TLC plate was Scanned under the 366 nm and 254 nm, and used for the photo documentation (Fig. 3 and 4). Rf value of the Quercetin and rutin were found to be 0.51 and 0.04 respectively (Fig. 5 and 6). The HPTLC profiling showed the presence of 8 and 7 spot in the methanolic extract of bark and leaf respectively (Fig. 7 and 8). Chromatogram analysis of methanolic bark extract showed that the max Rf value of the first peak and fifth peak coinciding with Rutin and Quercetin respectively (Table 2, 3 and 4). The max Rf value of the first and third peak of methanolic extract of leaf was also coinciding with the standard rutin and quercetin (Table 2, 3 and 5).

Previous papers demonstrated that HPTLC method can be used for the simultaneous quantitative determination of quercet in and rutin in *Catharanthus roseus* and *Triphala churna* leaves, mainly because of its simplicity, accuracy, and selectivity (**Rao and Ahme 2013, Pawar and Salunkhe, 2012**). HPTLC method is also the most suitable method for estimation of chemical constituents present in plant materials. Twobiologically active flavonoidal compounds quercetin and rutin estimated in *Tephrosia purpurea* etahanolic leaves extract using high-performance thin-layer chromatography (**Jain *et al.*, 2009**). Mobile phase was methanol-water-formic acid (40:57:3, v/v/v). HPTLC method developed for simultaneous qualitative and quantitative analysis of quercetin and rutin in the extract of *Saraca asoca* was found to be simple, specific, accurate, sensitive. It can be used as quality control of quercetin and rutin in the extract of *Saraca asoca* (**Prajapati *et al.*, 2013**).

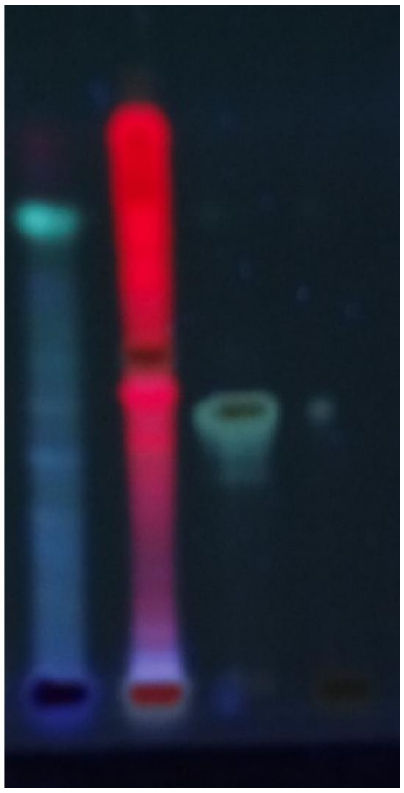

**Fig. 3. Photo documentation of *O. oojeinensis*at 366 nm**

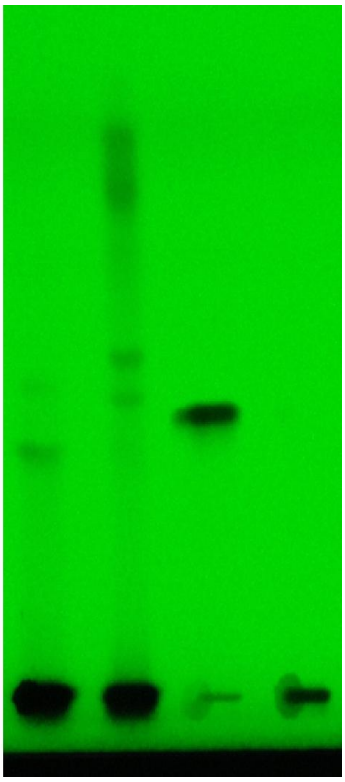

**Fig.4. Photo documentation of *O. oojeinensis* at 254 nm**

**Table 2. CAMAG TLC Scanner Analysis Report of Quercetin**

| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.01 | 44.1 | 0.04 | 253.9 | 23.56 | 0.08 | 4.4 | 5057.3 | 12.33 | unknown * |
| 2 | 0.36 | 17.2 | 0.51 | 823.9 | 76.44 | 0.56 | 2.2 | 35973.8 | 87.67 | unknown * |

**Table 3. CAMAG TLC Scanner Analysis Report of Rutin**

| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.01 | 155.0 | 0.04 | 658.0 | 84.32 | 0.09 | 1.6 | 23105.5 | 89.27 | unknown * |
| 2 | 0.45 | 4.6 | 0.51 | 106.9 | 13.70 | 0.54 | 0.9 | 2679.2 | 10.35 | unknown * |
| 3 | 0.83 | 3.7 | 0.84 | 15.5 | 1.98 | 0.85 | 1.2 | 98.2 | 0.38 | unknown * |

**Table 4. CAMAG TLC Scanner Analysis Report of bark extract**

| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.00 | 16.9 | 0.04 | 607.0 | 78.04 | 0.09 | 6.2 | 20609.4 | 82.22 | unknown * |
| 2 | 0.17 | 4.8 | 0.18 | 14.6 | 1.87 | 0.19 | 0.1 | 117.4 | 0.47 | unknown * |
| 3 | 0.28 | 0.9 | 0.34 | 10.7 | 1.38 | 0.37 | 4.0 | 299.0 | 1.19 | unknown * |
| 4 | 0.40 | 10.1 | 0.45 | 50.1 | 6.44 | 0.48 | 25.9 | 1639.7 | 6.54 | unknown * |
| 5 | 0.48 | 26.4 | 0.50 | 49.5 | 6.36 | 0.52 | 18.6 | 1123.9 | 4.48 | unknown * |
| 6 | 0.54 | 11.9 | 0.55 | 16.2 | 2.08 | 0.58 | 1.3 | 316.6 | 1.26 | unknown * |
| 7 | 0.69 | 3.6 | 0.74 | 18.8 | 2.42 | 0.77 | 6.5 | 698.8 | 2.79 | unknown * |
| 8 | 0.90 | 2.9 | 0.93 | 10.9 | 1.40 | 0.95 | 8.1 | 259.8 | 1.04 | unknown * |

**Table 5. CAMAG TLC Scanner Analysis Report of leaf extract**

| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.01 | 99.0 | 0.04 | 548.8 | 38.97 | 0.11 | 0.1 | 17626.9 | 38.77 | unknown * |
| 2 | 0.39 | 10.5 | 0.45 | 77.0 | 5.47 | 0.48 | 41.7 | 2827.5 | 6.22 | unknown * |
| 3 | 0.50 | 50.1 | 0.54 | 241.0 | 17.12 | 0.57 | 86.8 | 6170.5 | 13.57 | unknown * |
| 4 | 0.57 | 87.5 | 0.61 | 325.3 | 23.10 | 0.65 | 0.2 | 9069.4 | 19.95 | unknown * |
| 5 | 0.71 | 2.8 | 0.74 | 20.4 | 1.45 | 0.77 | 2.3 | 444.3 | 0.98 | unknown * |
| 6 | 0.77 | 3.1 | 0.80 | 15.0 | 1.07 | 0.81 | 9.1 | 324.5 | 0.71 | unknown * |
| 7 | 0.82 | 8.4 | 0.89 | 180.7 | 12.83 | 0.93 | 137.3 | 8999.7 | 19.80 | unknown * |

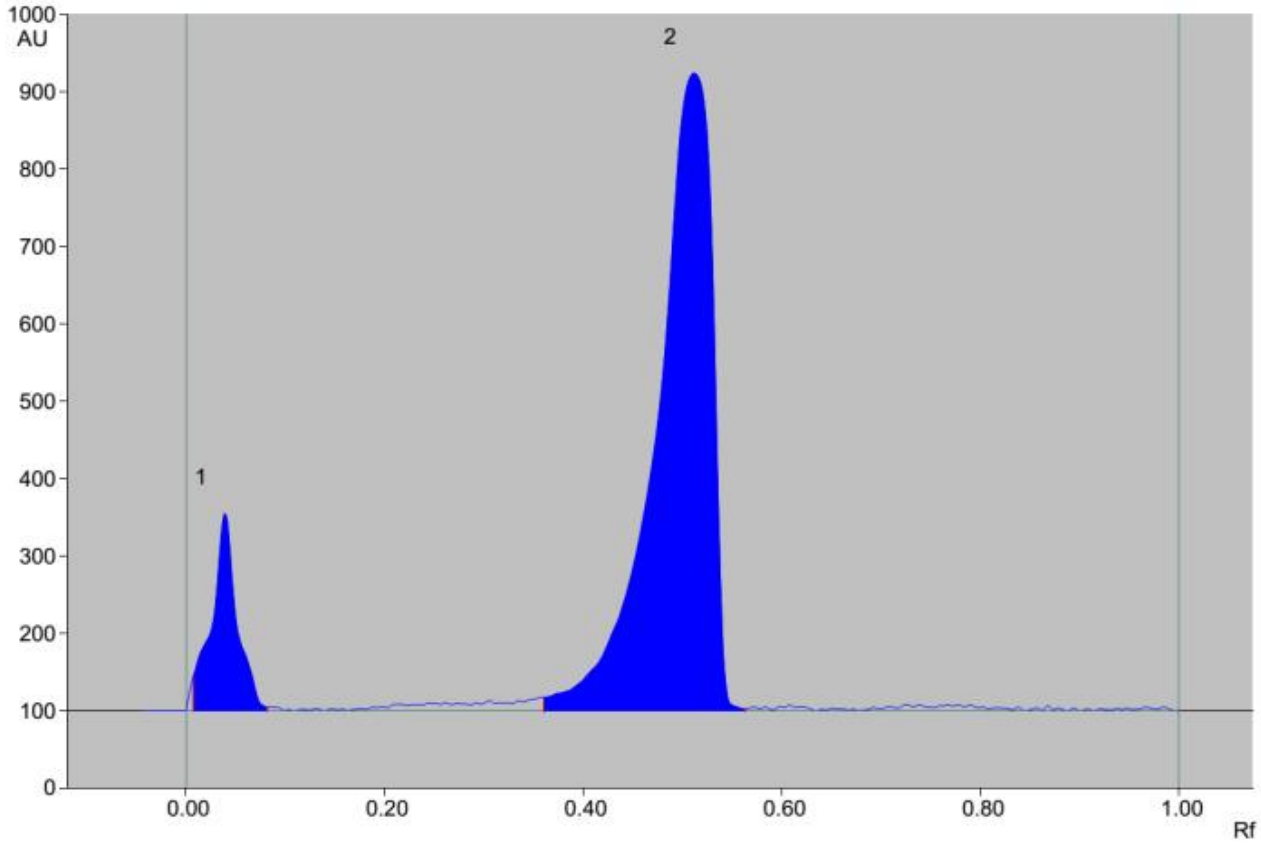


**Fig.7. CAMAG TLC scanner graph of Quercetin**

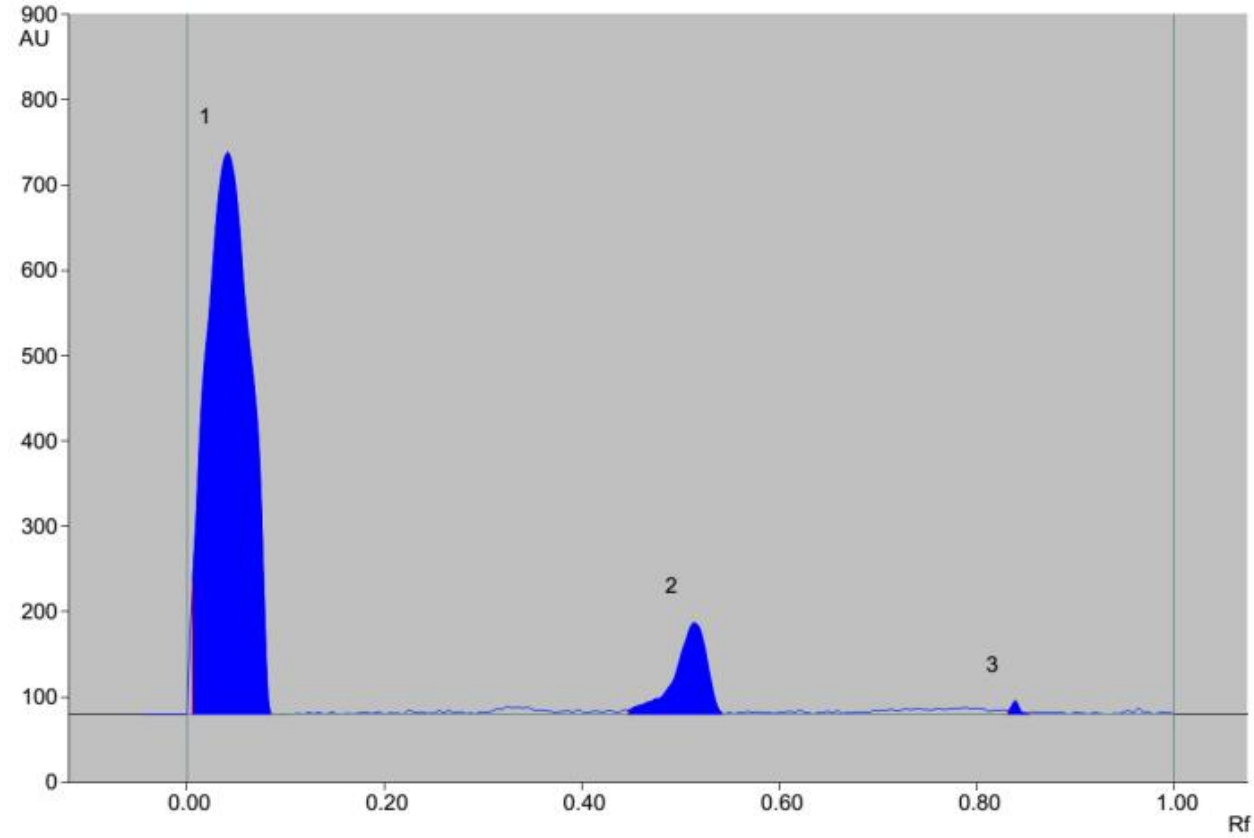


**Fig.8. CAMAG TLC scanner graph of Rutin**

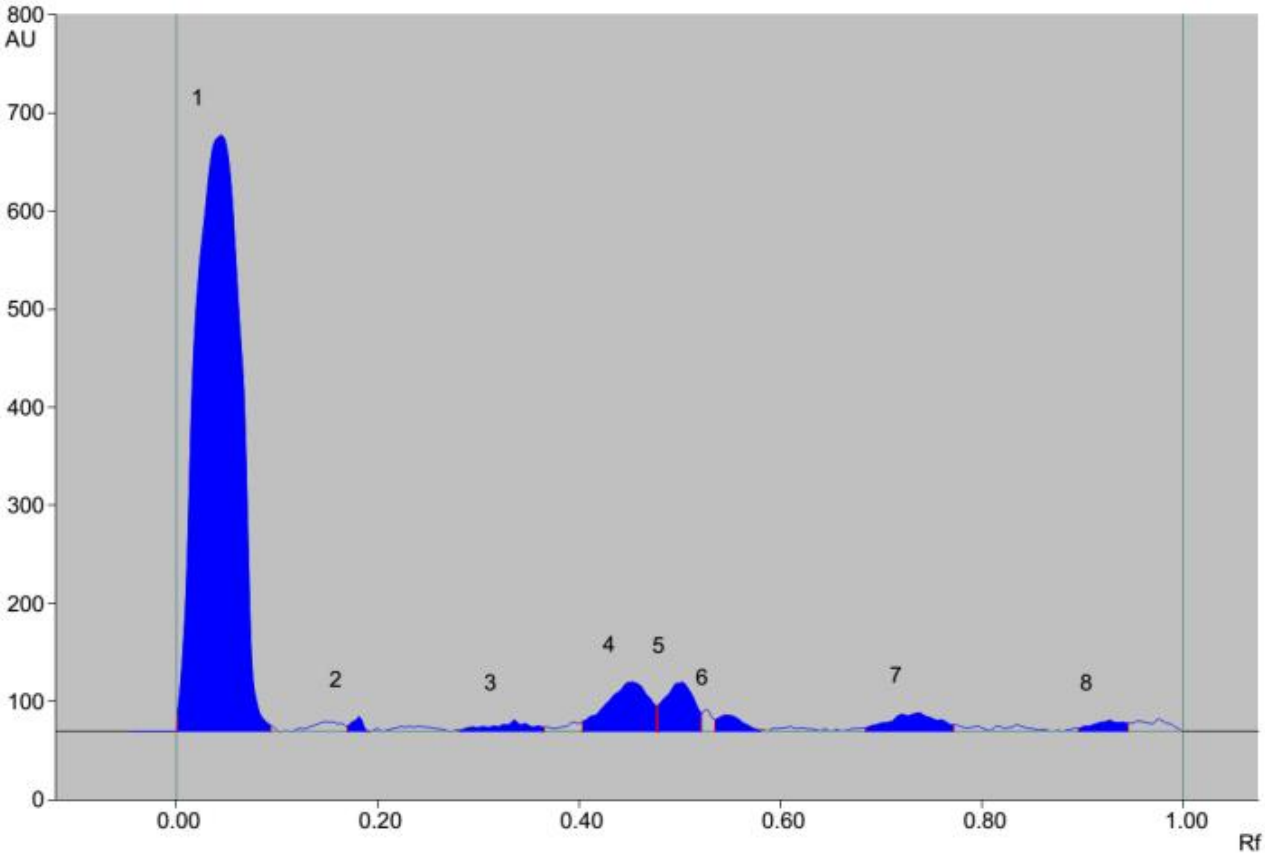


**Fig.5. CAMAG TLC scanner graph of bark extract**

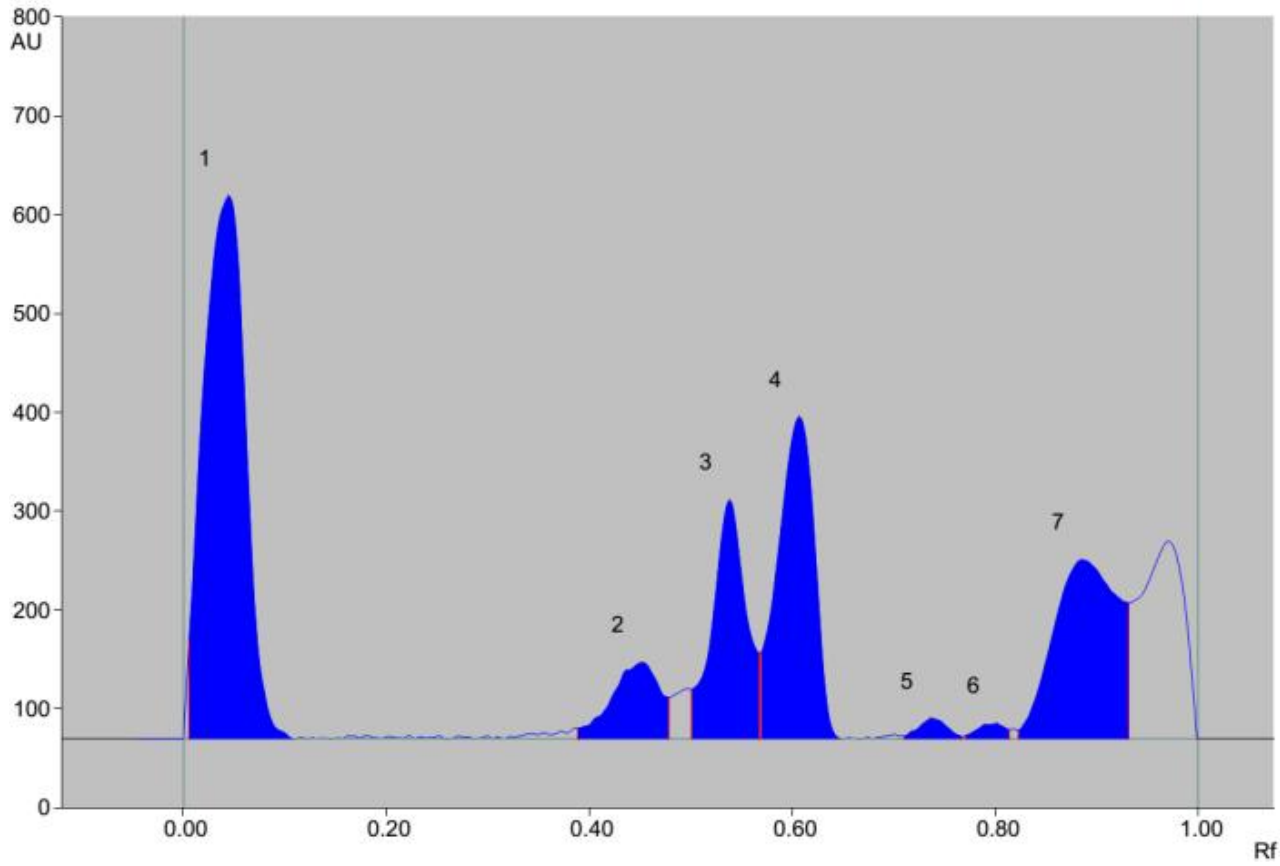


**Fig.6. CAMAG TLC scanner graph of leaf extract**

Successfully and also thankful to UGC for sanctioning FIP. HPTLC fingerprinting profile is very important parameter of herbal drug standardization for the proper identification of medicinal plants (**Tambe *et al.*, 2014**). This is the first time method was developed for the estimation of rutin and quercetin from the leaf and bark methanolic extracts of *Ougeinia oojeinensis.*

## Conclusion

Present study showed that the methanolic leaves and bark extract of *Ougenia oojeinensis* (Roxb.) Hochr. contain carbohydrate, saponin, tannins, flavonoids, steroids, alkaloids, glycosides, terpenoids and protein. HPTLC is feasible for development of chromatographic fingerprints to determine major active constituents of medicinal plants. It provides a rapid, easy, accurate and specific HPTLC method for quantitative estimation rutin and quercetin in the *Ougeinia oojeinensis* bark and leaf metanolic extract. Such finger printing is useful in differentiating the species from the adulterant and act as a biochemical marker for this medicinally important plant in the pharmaceutical industry and plant systematic studies. The analysis shows that the plant is rich in secondary metabolites which could be explored as potential drug in phytomedicine.

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