Vol 9|Issue 2| 2019 | 29-33.

e-ISSN 2248 - 9169
Print ISSN 2248 - 9150

International Journal of
Experimental Pharmacology

www.ijepjournal.com

ISOLATION AND CHARACTERIZATION OF RUTIN FROM TAGETES ERECTA L.

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ABSTRACT

Tagetes erecta L. is a widespread garden plant that is commonly known as the marigold, and it is widely used as a medicinal herb for its anti-inflammatory, analgesic, and anti-edematous properties, which are important for phytotherapeutic, dermatological and cosmetic applications. Rutin is that the major flavonoid glycoside found in gingko is that the rhamno glucoside of the flavonoid quercetin has been referred to as vitamin P or the porousness issue. Many studies had been done for the isolation of rutin by completely different chromatographically methodology. During this study rutin was isolated Tagetes erecta L. by precipitation and fractional solubilization. The isolated rutin was known by measure its melting point, ultraviolet absorption, FTIR spectra, HPLC and TLC.

INTRODUCTION

Tagetes erecta L. is a widespread garden plant that is commonly known as the marigold, and it is widely used as a medicinal herb for its anti-inflammatory, analgesic, and anti-edematous properties, which are important for phytotherapeutic, dermatological and cosmetic applications [1,2]. Essential oil from marigold has been shown to be an effective free radical scavenger, and the ethanol extract is reportedly effective against parakeratosis [3]. This plant is rich source of alkaloids, volatile oils and flavonoids [4]. Flavonoids, a group of natural substances with variable phenolic structures, are found in fruits, vegetables, grains, bark, roots, stems, flowers, tea and wine. These natural products are well known for their beneficial effects on health and efforts are being made to isolate the ingredients so called flavonoids. Flavonoids are now considered as an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. This is attributed to their anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function[5]. The antioxidant activity of flavonoids depends upon the arrangement of functional groups about the nuclear structure. The configuration, substitution, and total number of hydroxyl groups substantially influence several mechanisms of antioxidant activity such as radical scavenging and metal ion chelation ability[6].

Rutin (3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoglucoside) is a flavonol, abundantly found in plants, such as passion flower, buckwheat, tea, and apple. It is a vital nutritional component of food stuff. Rutin, also called as rutoside, quercetin-3-rutinoside, and sophorin is a citrus flavonoid glycoside found in buckwheat. The name ‘rutin’ comes from the plant Ruta graveolens, which also contains rutin. Chemically it is a glycoside comprising of flavonolic aglycone quercetin along with disaccharide rutinose. It has demonstrated a number of pharmacological activities, including antioxidant[7], antidiabetic [8], cytoprotective, vasoprotective, anticarcinogenic, neuroprotective [9,10] and cardioprotective activities[11-15]. The present work is aimed to isolate and characterize rutin from *Tagetes erecta* L.

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EXPERIMENTAL

Chemicals

Petroleum ether, ethanol, ethyl acetate and methanol were purchased frm Central Drug House, India. All the other chemicals used in the study were of analytical grade.

Isolation of Rutin

Twenty grams of the powdered *Tagetes erecta* L leaves were extracted by Soxhlet apparatus with 250 ml of 80% ethanol until exhaustion. The extract was filtered and targeted by evaporation underneath vacuum to regarding 10 ml then mixed with 25 ml H_2O, and extracted with petroleum ether (50 ml x 3), then with chloroform (50 ml x 3). After extraction, the aqueous layer was collected and left to stand during a cold place for 72 hours; a yellow precipitate separated out of the solution. The precipitate was filtered and washed with a combination of chloroform: ethyl acetate: ethanol (50:25:25). The un-dissolved a part of the precipitate was dissolved in hot methanol and filtered, the filtrate was evaporated to dryness to present 100 mg yellow powder (Rutin), and its melting point was measured.

Yield and Melting Point

The yield of isolated compound was calculated and its melting point was determined.

HPLC Analysis

The isolated rutin was known by HPLC technique and compared with standard rutin using column and a combination of methanol: water (1:1 ratio) as a mobile phase with a flow rate of 1 ml min-1and detected 360 nm.

TLC and Paper Chromatography

Isolated rutin was conjointly compared with standard rutin using TLC method; a pre-coated aluminum sheet with silica gel G with the subsequent mobile phases: ethyl acetate: butanone: formic acid: water (50:30:10:10), ethyl acetate: formic acid: acetic acid: water (100:11:11:27). In paper chromatography, Watman No.1 filter paper was used as a stationary phase and mobile phases of: acetic acid: water (15:85) and isopropyl alcohol: water (60:40).

Spectrophotometric Analysis

The isolated rutin was dissolved in methanol and its ultraviolet radiation absorption peaks were determined and compared with standard rutin. Infrared spectrum of the isolated rutin was determined using KBr disk methodology.

RESULT AND DISCUSSION

The yield was found to be 523 mg. The isolated compound demonstrated a melting point at a range of 180-189 ℃ which is identical with that reported, In TLC and Pc study, R_f of isolated compound was near to Rutin (Table 1).

Table 1: Comparison between the R_F Values of Isolated and Standard Rutin in Different Mobile Phase

Technique	Solvent system in	Rf value of isolated rutin	Rf value of standard rutin
Thin Layer Chromatography	Ethyl acetate : formic acid : acetic acid : water (1:1:1:1)	0.35	0.34
Paper chromatgraphy	Isopropyl alcohol : water (1:1)	0.58	0.59

For rutin, the HPLC chromatograms of the isolated were within the desired retention time (fig 2).

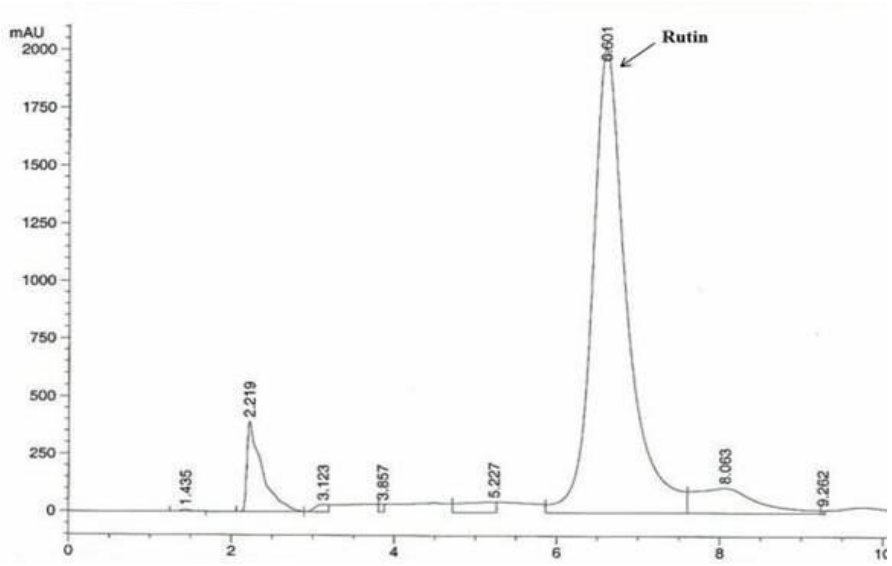

Figure 1: HPLC chromatogram of Rutin

UV-Visible spectra of isolated compound and rutin were at the affinity. The spectrum of rutin showed 2 major absorption bands at 369, 364 nm that indicated the presence of flavonol structure. the primary absorption most will be thought of as originating from π-π* transitions with in the ring A (aromatic system) and also the second absorption most determined around 364 nm, which can be assigned to transitions in ring B (cinnamayl system)

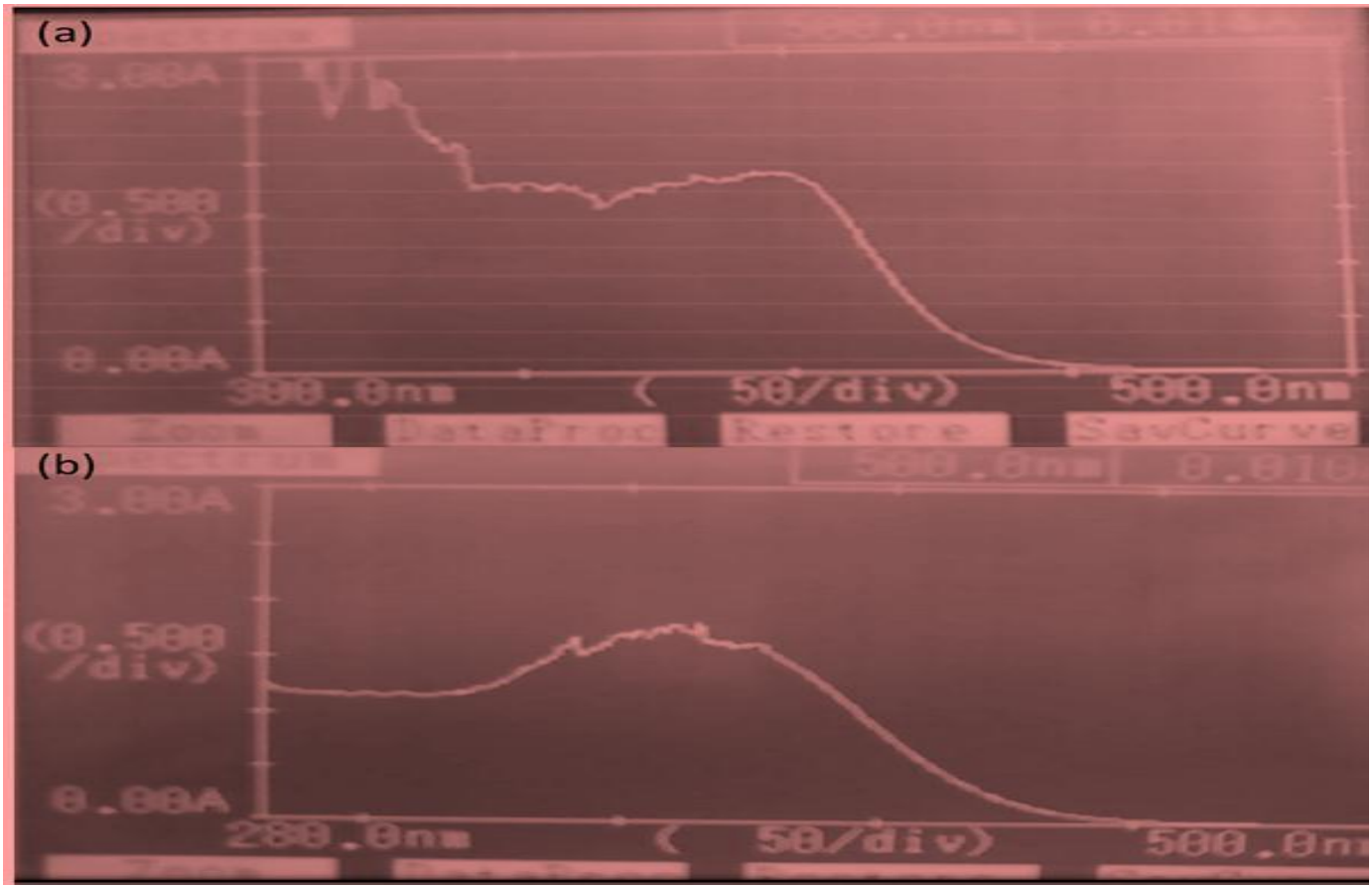

Figure 2: UV visible spectra of (a) isolated compound (b) rutin

In case of FTIR, the characteristic frequencies as in case of Aromatic C=C Bending, C – H bending, C=C Stretching.

Table 2: Characteristic frequencies of isolate compound (rutin)

Intensities (cm^{-1})	**Functional Group**
3294.01	N-H stretching
1672.40	Asymmetric stretching
1550.41	Aromatic C=C Bending
1458.26	C – H bending
1317.17	Ring structure
1244.34	C-N
1166.11	C=C Stretching
1095.22	C-O stretching
933.61	Symmetric stretching
637.06	-CH=CH-(cis)

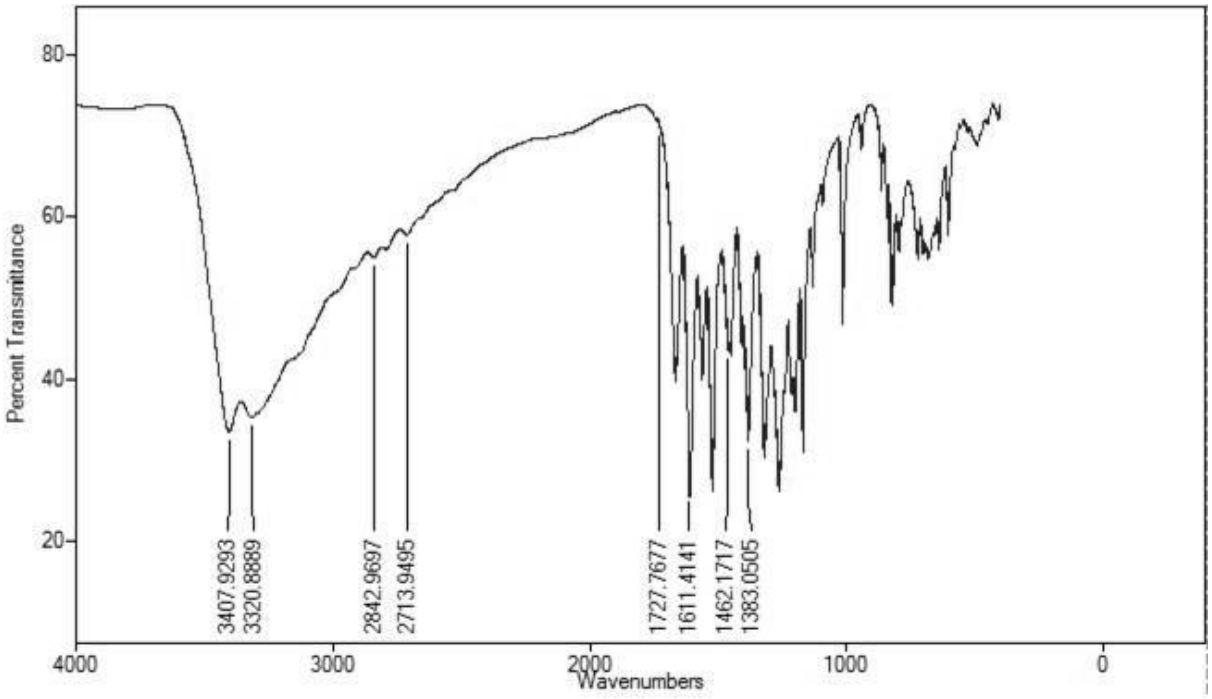

Figure 3: FTIR spectra of Rutin

CONCLUSION

The flavonoid Rutin was isolated & purified from natural sources depending on the variations in solubility compared to its aglycone part significantly once. Rutin is that the major glycoside found within the plant.

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