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HPLC Analysis and Isolation of Rutin from Stem Bark of *Ginkgo biloba* L.

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ABSTRACT

Ginkgo biloba L., a plant belongs to the Ginkgoaceae is characterized by a very wide spectrum of various plant constituents. 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 from *ginkgo bilobo* by precipitation and fractional solubilizations while not the utilization of any chromatographical technique. The isolated rutin was known by measure its melting point, ultraviolet absorption, FTIR spectra, HPLC and TLC.

Keywords: *Ginkgo biloba*, Flavonoids, Isolation, Rutin

1. Introduction

Ginkgo biloba is one in all the most well-liked useful plants, particularly as medicinal plants. Extracts of *G. biloba* leaves contain active compounds like flavonoids and terpene lactones (ginkgolides and bilobalides) and may so be used to increase peripheral and cerebral blood flow [1, 2]. So far, concerning 38 varieties of flavonoids are isolated from *Ginkgo biloba.*

Ginkgo biloba was grown throughout China and Korea, and was introduced into Japan concerning 800 years ago, then into Europe around 1730, and to North America in 1784. The term "*Ginkgo"* was initial utilized by the German physician and botanist Engelbert Kaempferol in 1712, however Linnaeus provided the bionomical word "*Ginkgo biloba*" in 1771, "biloba" which means two-lobed (from Latin bi: double, Loba: lobes), concerning the fan-shaped leaves split within the middle [3].

The two main pharmacologically active teams of compounds present within the *Ginkgo biloba* leaf extract are the flavonoids and therefore the terpenoids. Flavonoid also referred to as phenylbenzopyrones or phenylchromones are a group of low molecular weight substances that are wide unfold within the plant kingdom. Flavonoid present within the *Ginkgo biloba* leaf extract are flavones, flavonols, tannins, biflavones (amentoflavone, bilobetol, 5-methoxybilobetol, ginkgetin, isoginkgetin and sciadopitysin), and associated glycosides of quercitin and kaempferol attached to 3-rhamnosides, 3-rutinosides, or p-coumaric esters [4].

Two types of terpenoids are present in *Ginkgo biloba* as lactones (non saponifiable lipids present as cyclic esters): ginkgolides and therefore the bilobalide. Ginkgolides are diterpenes with 5 types A, B, C, J, and M, wherever types A, B, and C account for around 3.1% of the overall *Ginkgo biloba* leaf extract [5]. Bilobalide, a sesquiterpene trilactone, accounts for the remaining 2.9% of the overall standardized *Ginkgo biloba* leaf extract. *Ginkgo biloba L.* extracts (GBE) are usually utilized in Europe for the treatment of cerebrovascular and peripheral circulatory problems of the elderly [6]. These extracts contain flavonol glycosides [7], biflavones, and terpenes. The pharmacologically active components, the diterpenes Ginkgolide A, B, and C (GA, GB, and GC) and therefore the sesquiterpene bilobalide (BB), are reportable to be solely present in *Ginkgo biloba* L. Thus, the contents of GA, GB, GC, and BB are usually used because the quantitative indices of GBE, and therefore the analysis of those constituents are of great importance in production process control [8].

Ginkgo biloba could also be of great value in cases of age-related mental dysfunction as well as senility, Alzheimer's disease and diminished memory. Rutin is that the rhamno glucoside of the flavonoid quercetin, and located in several plants and used for treatment of varied diseases associated with the vascular [9]. Its quercetin-3-rutinoside or 3, 3', 4', 5, 7-pentahydroxy flavones-3-rutinoside and has a chemical formula $C_{27}H_{30}O_{16}$.

Major commercial sources of rutin include Saphora japonica, Eucalyptus spp., fagopyrum esculatum, ruta grave lens and *ginkgo biloba*. As a constituent of fagopyrum esculatum, British European pharmacopoeias need a minimum content of 4.0% flavonoids determined by liquid chromatography with absorbance at 350 nm [10].

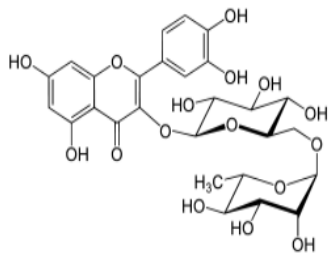

Fig 1: Chemical structure of Rutin

Rutin (Fig.1) and quercetin were isolated from tobacco leaves by column chromatography and preparative TLC [11] and from Ruta grave lens and alternative plants using chromatographic techniques and inaudible methods [12]. This study was designed to isolate rutin from ginkgo based on its solubility in different solvents with none alternative separation techniques.

2. Material and Methods

HPLC, UV-visible spectrophotometer (Shimadzu 1700), FTIR. All chemicals and solvents are of analytical grade. Standard rutin was obtained from Loba Chemie Company.

2.1 Plant Material

The leaves & stem bark of *Ginkgo biloba* were collected from FRI (Forest research Institute) in Dehradun and authenticated by the Botany division they were dried in shade for several days at room temperature and then grinded as powder.

2.2 Extraction and Isolation

Twenty grams of the powdered *Ginkgo biloba* (leaves & stem bark) part was 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 (50ml 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.

2.3 Identification of Isolated Rutin

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^{-1} and detected 360 nm.

2.4 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) [13].

2.5 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.

3. Result and Discussion

Isolated compound from stem bark and leaves showed a melting point at a range of 180-189 °C, 181-187 °C and 179-185 °C which is identical with that reported, For rutin, the HPLC chromatograms of the isolated rutin & standard rutin when extraction and isolation with chloroform and standard rutin are shown in (fig 4 and 5) respectively, whereas the R_f values of isolated and standard rutin in several mobile phases are shown in table 1.

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

Solvent system in TLC and Paper Chromatography	R_f value of isolated rutin	R_f value of standard rutin
TLC for leaves		
Ethyl acetate : formic acid : acetic acid : water	0.39	0.34
TLC for stem bark		
Ethyl acetate : formic acid : acetic acid : water	0.44	0.41
Paper chromatography for leaves		
Isopropyl alcohol : water	0.55	0.59
Paper chromatography for stem bark		
Isopropyl alcohol : water	0.71	0.75

The infrared spectrum is shown in (fig 6) whereas the ultraviolet spectra of isolated and standard rutin are shown in (fig 2, 3). 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); this band appeared

broad as a results of overlapping with LMCT band [14,15]. The principle of this technique depends on the differences in solubility between the glycoside and its aglycone. After concentrating the aqueous-alcoholic extract the solubility of the glycoside and the aglycone decrease and therefore they precipitates is further fractionated by dissolving the aglycone in the chloroform: ethylacetate: ethanol mixture leaving the glycosides which was later on dissolved in hot methanol, and so this method of isolation.

3.1 Spectrophotometric analysis

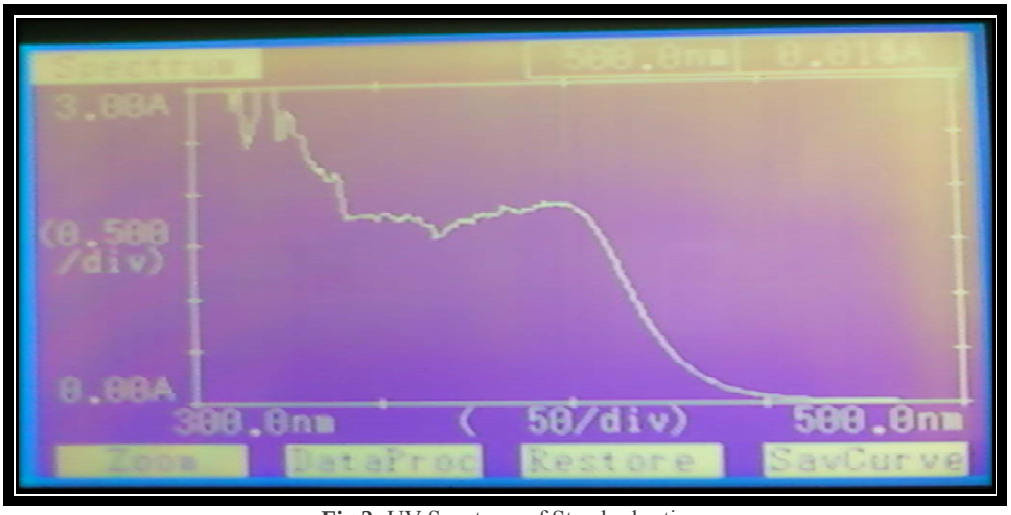

Fig 2: UV Spectrum of Standard rutin

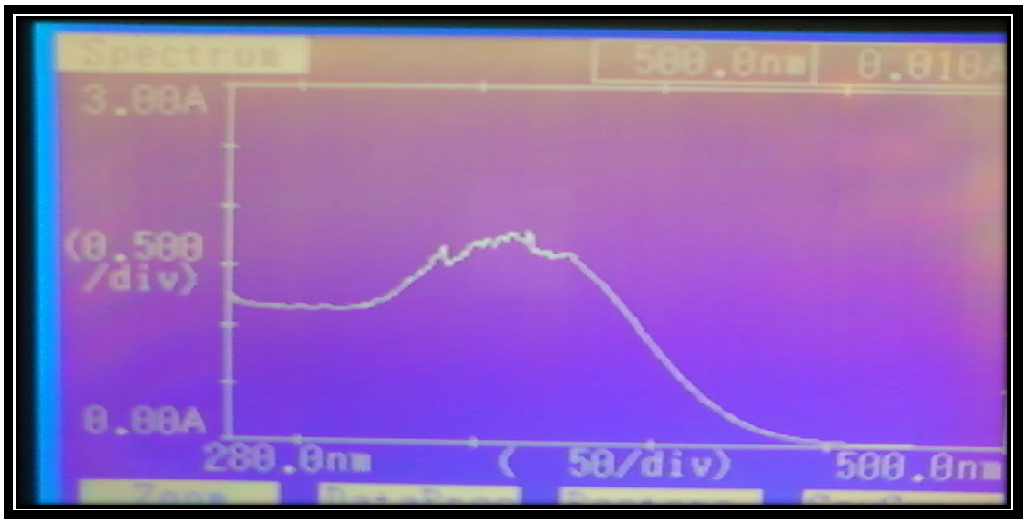

Fig 3: UV Spectrum of isolated rutin from stem bark

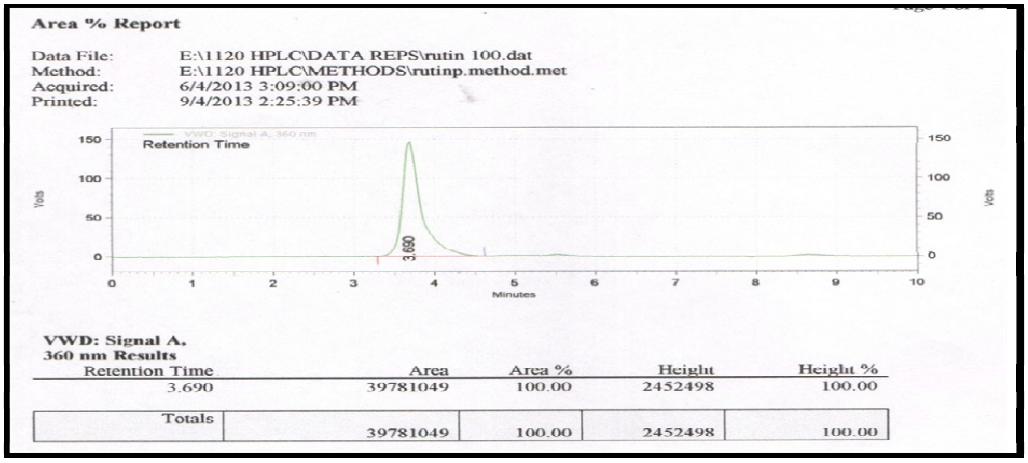

Fig 4: HPLC chromatogram of the standard Rutin

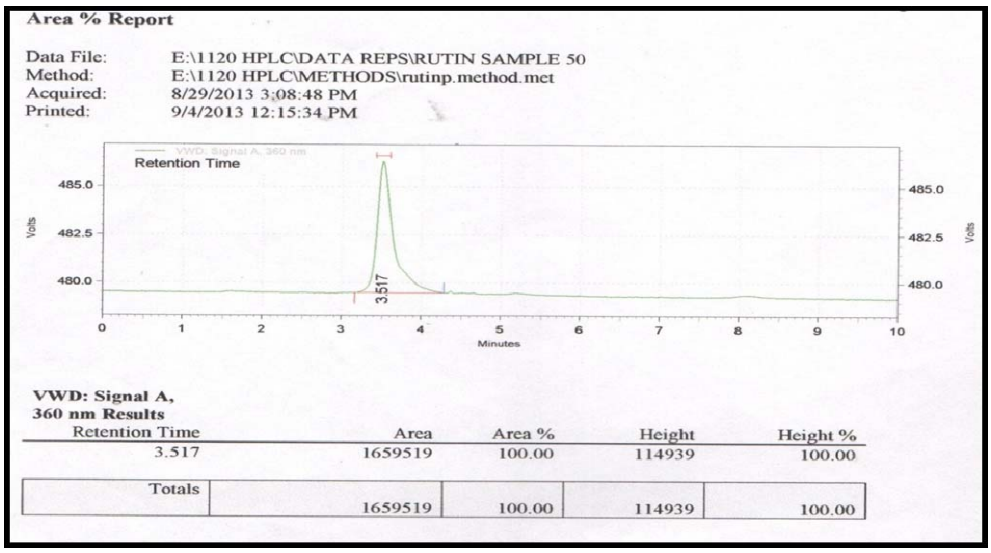

Fig 5: HPLC Chromatogram of the isolated rutin from stem bark

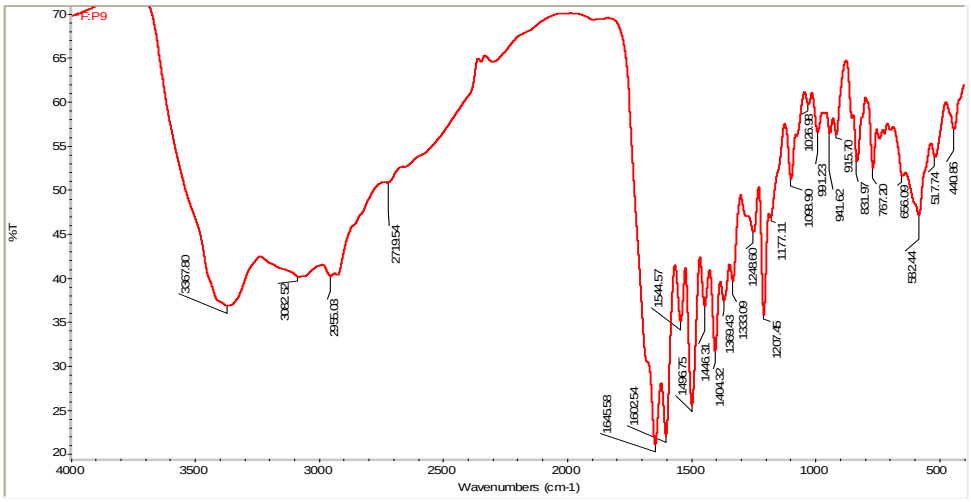

Fig 6: IR spectrum of the isolated rutin from stem bark

4. Conclusion

The flavonoid Rutin will be isolated & refined 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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