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PHYSICOCHEMICAL CHARACTERIZATION AND DETERMINATION OF FREE RADICAL SCAVENGING ACTIVITY OF RUTIN-PHOSPHOLIPID COMPLEX

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ABSTRACT

Rutin belongs to a class of plant secondary metabolites called flavonoids. Rutin is believed to be a vital nourishing supplement as it is able to make the capillaries stronger. But when administered orally it shows poor absorption because of less lipophilicity. To overcome this limitation, the present study was aimed to develop Rutin- phospholipid complex in different ratio to improve the lipophilic properties of rutin. The prepared complex was evaluated for physicochemical properties and *in-vitro* DPPH radical's scavenging activity. The physicochemical properties of the complex were analyzed by ultraviolet-visible spectroscopy (UV), infrared spectroscopy (IR) and X-ray diffractometry (XRD). The results showed that rutin-phospholipid in the complex were joined and did not form a new compound. Results of DPPH radical are scavenging activity revealed that among all ratios of complex, 1:3 next to this 1:2 followed by 1:1. The study was concluded that the free radical scavenging activity was observed in concentration dependent manner.

INTRODUCTION: Rutin (3, 3′, 4′, 5, 7-pentahydroxyflavone-3-rhamnoglucoside) [1] is a flavanol glycoside (**figure 1**) plant metabolite extracted from buckwheat and Japanese pagoda tree and is able to antagonize the increase of capillary fragility associated with hemorrhagic disease or hypertension [2].

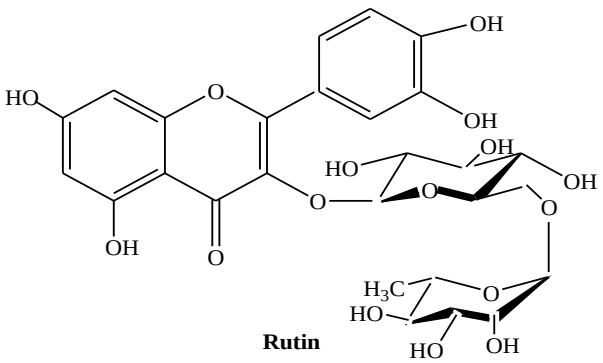


FIGURE 1: CHEMICAL STRUCTURE OF RUTIN

The important properties of rutin are significant scavenging properties on oxidizing species such as hydroxyl radical, superoxide radical, and peroxyl radical is assumed that the observed protective effects derive from the antioxidative and radical-scavenging properties [3].

Rutin is an oral capillary preservatory drug usually used for the therapy of chronic venous insufficiency and is also an ingredient in a large number of multivitamin preparations and herbal remedies [4].

The interest in using rutin in cosmetic and pharmaceutical formulations is to enhance their antioxidant and vasoprotective properties, promoting relief of the symptoms of lymphatic and venous insufficiency, and reducing capillary fragility.

It is worth considering that, being a non-toxic, nonoxidizable molecule, rutin offers an advantage over other flavonoids, which on some occasions behave as pro-oxidant agents and catalyse oxygen radical's production.

In previous studies, it has been mentioned that rutin posses lower lipophilicity which somewhat reduces its formulation development acceptance. Therefore, in this study, an attempt was taken to enhance the lipophilicity of rutin by preparing its complexes with phospholipid and comparatively evaluate the physicochemical and free radical scavenging property with rutin.

MATERIALS AND METHODS:

1. **Materials:** Rutin and phospholipid (soya lecithin) was purchased from Loba Chemie limited and 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Other chemical and reagents were of analytical grade.

2. **Preparation of rutin-phospholipid complex:** Rutin-phospholipid complex was prepared by anhydrous co-solvent lyophilization method. In briefly, rutin powder was dissolve in methanol and phospholipid was dissolve in methanol separately. Both are mix by gentle agitation until formation of a clear mixture. The resultant homogeneous solution was then freeze-dried under vacuum and stored in air tight container for further use [5]. For preparation of complex in the ratio of 1:1, 100 mg of rutin was dissolved in 10 ml of methanol separately and 100 mg phospholipid was dissolved in 10 ml chloroform, separately. Both the solution was mixed and stirred in mechanical stirrer up to methanol was completely evaporated. The residue was ground and the resultant powder was collected as rutin-phospholipid complex.

Characterization: The prepared rutin-phospholipid complex (1:1, 1:2 and 1:3) was subjected to structural analysis by UV spectroscopy, IR spectroscopy, and X-ray diffraction pattern.

1. **UV and IR analysis:** The UV absorption spectrum was recorded on Shimadzu-1700 UV/Visible Spectrophotometer. The FTIR absorption spectrum was recorded on FT-IR spectrophotometer (ABB, FTLA-2000, Japan)

2. **X-ray diffractometry (XRD):** X-ray diffraction pattern was recorded on Philips Analytical X-Ray BV1710. The powders of samples were packed tightly in a rectangular aluminum cell. The samples were exposed to the X-ray beam from an X-ray generator running at 36 kV and 20 mA. The scanning regions of the diffraction angle, 2θ, were 5-60°. Duplicate measurements were made at ambient temperature. Radiation was detected with a proportional detector.

***In-Vitro* Antioxidant Activity:**

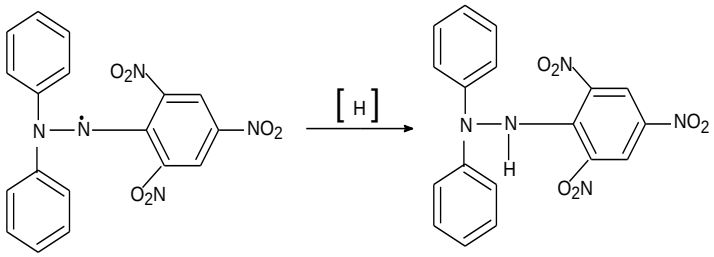
1. **DPPH Radical Scavenging Assay:** The DPPH free radical when it reacts with hydrogen donors (**figure 2**). Initially DPPH radical is purple and upon reaction with hydrogen donor's, it becomes colourless and formation of the non radical form of DPPH. It is a discoloration assay, which is evaluated by the addition of the antioxidant to a DPPH solution in methanol and the ability to scavenge the stable free radical of DPPH was measured in the absorbance at 517 nm [6].

    a. **Preparation of the Test Sample:** 10 mg of the sample was dissolved in 10 ml of the methanol to make a stock solution (1000 µg/ml) separately. Different concentrations 10, 20, 30, 40, 50, 60, 70, 80 and 90 µg/ml samples were prepared from stock solution (1000 µg/ml) separately. Aliquots sample was prepared by taking 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9 ml respectively from stock solution of 1000 µg/ml and diluted up to 10 ml with methanol in 10 ml volumetric flask separately.

    b. **Preparation of DPPH (0.1mM) Solution:** DPPH solution in the concentration of 0.1mM was prepared in methanol.

    c. **DPPH Radical Scavenging Activity:** To 2ml of various test samples, 2ml solution of DPPH 0.1mM was added separately. The reaction mixture was shaken and incubated in the dark for 30 min, at room temperature and the

absorbance was recorded at 517 nm against methanol. Controls containing methanol instead of the antioxidant solution, and blanks containing methanol instead of DPPH solution were also made. The experiment was performed in triplicate. The inhibition of the DPPH radical by the samples was calculated with reference to control absorbance. The percentage of DPPH radical scavenging activity was plotted against the sample concentration [6, 7].



1, 1-Diphenyl- 2- picryl hydrazyl **1, 1-Diphenyl- 2- picryl hydrazine**

FIGURE 2: CONVERSION OF DPPH FREE RADICAL INTO 1, 1-DIPHENYL- 2- PICRYL HYDRAZINE

RESULT AND DISCUSSION: The The Photomicrography of prepared Rutin-phospholipid complex is shown in **Figure 3**. The UV spectra of rutin and their phospholipid complex are shown in **Figure 4**. The characteristic absorption peaks of rutin (271 nm) were still present. The infrared spectra of phospholipid, rutin and complexes are shown in **Figure 5**. The powder X-ray diffraction patterns of rutin and phospholipid complexes are shown in **Figure 6**.

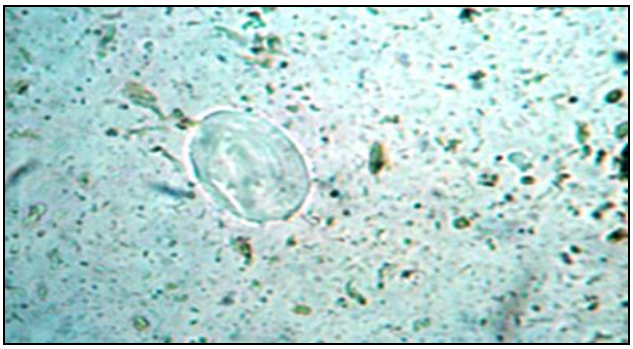


FIGURE 3: PHOTOMICROGRAPHY OF PREPARED RUTIN-PHOSPHOLIPID COMPLEX

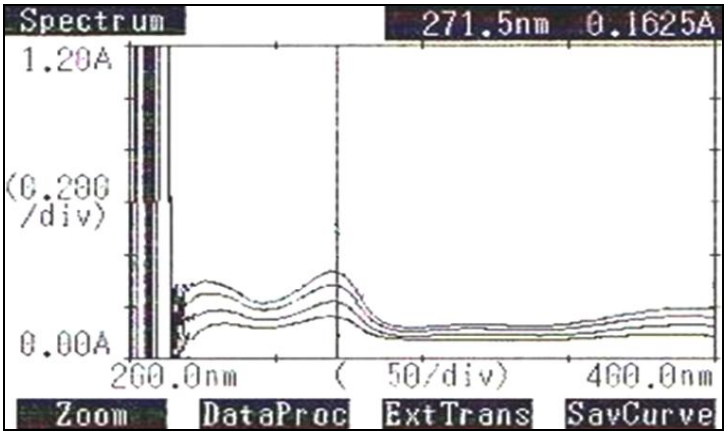


FIGURE 4: OVERLAY UV SPECTRA OF RUTIN AND RUTIN-PHOSPHOLIPID COMPLEX (1:1, 1:2 AND 1:3)

The powder diffraction pattern of rutin displayed sharp crystalline peaks, which is the characteristic of an organic molecule with crystallinity. In contrast rutin-phospholipid showed an amorphous state lacking crystalline peaks. This suggested that rutin in the phospholipid lipid matrix was either molecularly dispersed or in an amorphous form.

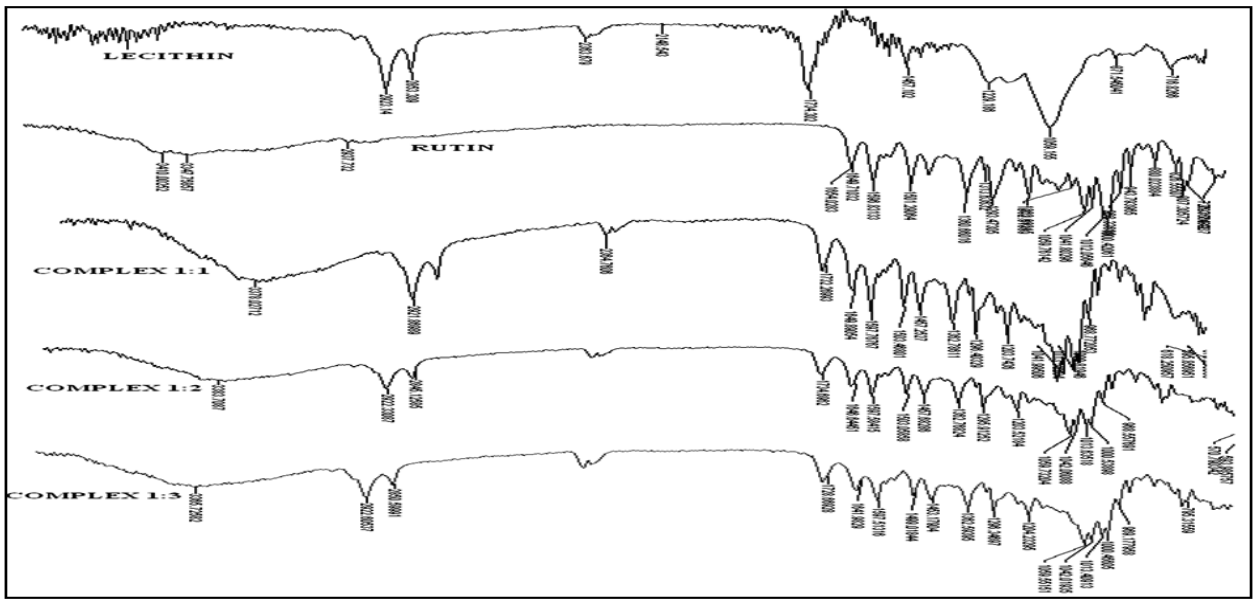


FIGURE 5: IR SPECTRA OF PHOSPHOLIPID AND RUTIN-PHOSPHOLIPID COMPLEX (1:1, 1:2 AND 1:3)

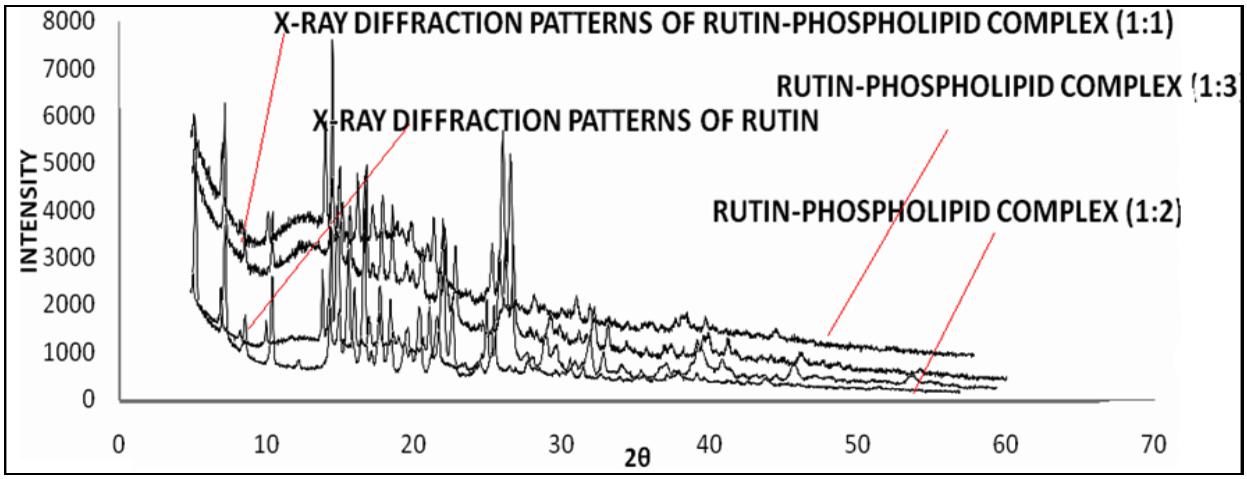


FIGURE 6: X-RAY DIFFRACTION PATTERNS OF RUTIN AND RUTIN-PHOSPHOLIPID COMPLEX (1:1, 1:2 AND 1:3)

In DPPH radical scavenging activity the DPPH radical is a stable organic free radical with an adsorption and at 517 nm. It loses this adsorption when accepting an electron or a free radical species, which results in a visually noticeable discoloration from purple to yellow. Among all prepared complexes, 1:3 exhibited maximum free radical scavenging activity, next to this 1:2 followed by 1:1 in comparison to rutin. This result was concluded that the free radical scavenging activity in concentration dependent manner (**Figure 7**).

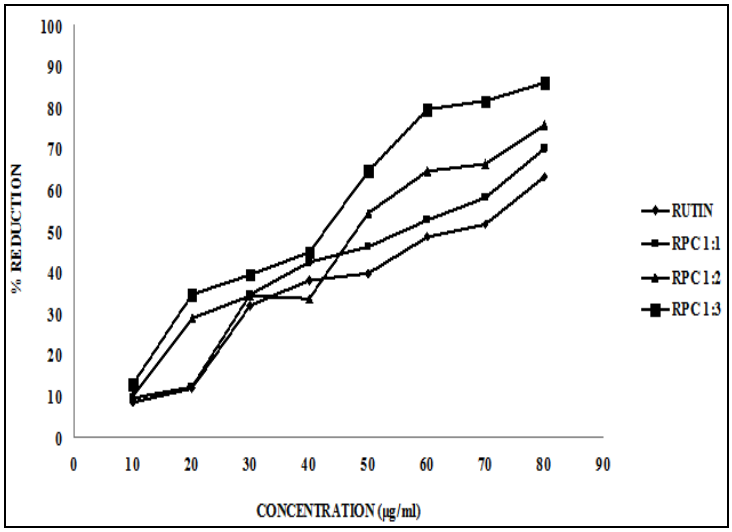


FIGURE 7: FREE RADICAL SCAVENGING ACTIVITY OF RUTIN AND RUTIN-PHOSPHOLIPID COMPLEX (1:1, 1:2 AND 1:3)

CONCLUSION: Rutin is a potent antioxidant found in many plants and vegetables. We tried to enhance the free radical scavenging property of this molecule through rutin-phospholipid complexes. The complexes were tested for its physicochemical properties and *in-vitro* antioxidant activity. The results obtained, proved better efficiency in the form of complexes as compared to the molecules itself.

The methanol was used as a reaction medium, rutin and rutin-phospholipid complexes were dissolved in the medium and after the organic solvent were removed, and the rutin–phospholipid complexes could be obtained. Using UV, IR and XRD, it could be concluded that rutin and rutin-phospholipid complex were joined by non-covalent-bonds, and did not form a new compound. The obtained complexes (1:3) showed strong antioxidant activity.

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