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Identification of Flavonoids (Quercetin, Gallic acid and Rutin) from *Catharanthus roseus* Plant Parts using Deep Eutectic Solvent

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Abstract
Green technology is the most important topic in the pharmaceutical field because it reduces the cost of medicines and minimizes the environmental impact of the field and is better for human health and safety. Green chemistry emphasizes that the solvent should be nontoxic, safe, cheap, green, readily available, recyclable, and biodegradable. Deep eutectic solvents, a new type of green solvent, have some renowned properties—for instance, high thermal stability, low vapor pressure, low cost, biodegradability, and high viscosity. In this study, deep eutectic solvents made up of choline chloride-glycerol (1:2) were used for the extraction and isolation of flavonoid (rutin, gallic acid, and quercetin) from *Catharanthus roseus* plant parts, flower petal, leaves, stem, and root. The amounts of rutin and quercetin in flower petal are 29.46 and 6.51%, respectively, whereas, rutin, gallic acid, and quercetin amounts in leaves are 25.16, 8.57, and 10.47%, respectively. In stem the amounts of rutin, gallic acid, and quercetin are 13.02, 5.89, and 7.47%, respectively. In root, only quercetin has been obtained that is 13.49%. The HPLC is an analytical method, which was found to be an excellent technique for determination of rutin, gallic acid, and quercetin using deep eutectic solvent extraction from plant parts of *Catharanthus roseus*.

Keywords: *Catharanthus roseus*; Deep eutectic solvent; Antioxidant activity; Flavonoids; HPLC.

1. INTRODUCTION

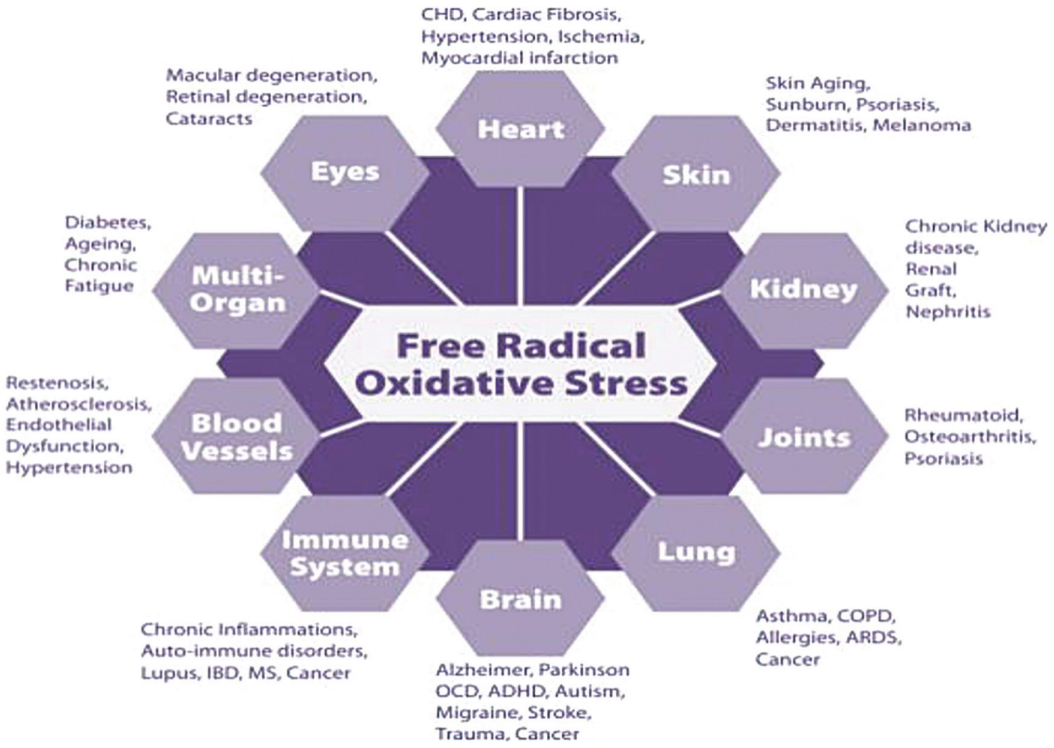
Solvents are most important for any type of extraction because the selection of solvent is directly related to the environment and the most important to the human health. Non-green solvent not only damages the environment but also destroy the human health. Conventional organic solvents, such as chloroform, hexane, ethyl acetate, acetone and methanol, were widely used for preparing bioactive active components from the medical plant [1]. Thus, a wide range of solvents of different polarities is required for the separation, extraction, purification, and administration of various chemicals. So far, alcohols, chloroform, and ethyl acetate are generally applied to this purpose. However, the use of large amounts of organic solvents may cause severe pollution of the environment and result in organic impurities in extracts, requiring special assays in quality control of extracts [2].

Ionic liquids (ILs) have found applications in very diverse areas and serve very different purposes. They can be used, for example, as extractions solvents [3, 4], as solvents for biocatalytic processes [5] and for electrochemical applications [6]. However, the application of synthetic ILs as solvents for extraction in pharmaceutical industry is limited because of high toxicity of some ingredients [7], their irritation properties, high costs of synthesis of the components, and tedious preparation procedures. Ils have been used for extracting some active compounds from plant materials including alkaloid [8], phenolic compounds [9], essential oils [10], and shikimic acid [11].

Deep eutectic solvents (DESs) are another type of solvents with similar physical properties [12]. An alternative to ILs is DESs, which may also have an ionic character but consist of a mixture of organic compounds having a melting point significantly lower than that of either individual component [13]. The most common DESs are based on choline chloride (ChCl), carboxylic acids, and other hydrogen-bond donors, for example, urea, succinic acid, citric acid, and glycerol. DESs have similar characteristics to ILs but are cheaper to produce (lower cost of the raw materials), less toxic, and often biodegradable [14]. DESs are a simple, green, and effective extraction method that has been used in this study. The alternative of harmful organic solvents is DESs, which is an effective method for the extraction of rutin, gallic acid, and quercetin from *Catharanthus roseus* plant parts. This study provides evidence on the extraction capacity of DESs. In this study choline chloride-glycerolbased DES is used, and it gave a favorable result for the extraction of rutin, gallic acid, and quercetin. Nam *et al.* [15] has used the DESs (choline chloride-glycerol) and based on observations, choline chloride-glycerol based solvents were much more efficient for the extraction of flavonoid from Flos sophorae. Chen *et al.* [16] described that the formation of hydrogen bond between glycerol and choline chloride can significantly improve the stability of salvianolic acid B from radix Salviae miltiorrhizae. Different choline chloride and glycerol (ChCl-GL) ratios were compared, including ChCl-GL (1:1, 1:2, 1:3, 1:4). The results illustrate that salvianolic acid B degradation trends were mainly the same in all given ratios of DESs, but ChCl-GL (1:2) ratio has been selected as solvent with a minor advantage for storing salvianolic acid B. Overall, ChCl-GL (1:2) ratio has given slight advantage then the remaining ones.

For the last few years, oxidative stress–related diseases such as neurodegenerative, cardiovascular, mitochondrial, metabolic diseases and even cancer have gained special attention [17]. Free radical oxidative stress causes many diseases and disorder that are given below in Figure 1.

Figure 1: Free radical oxidative stress.



The study of the antioxidant potential of phenolic extracts derived from plant species is one of the hot topics in the scientific community; however, in vitro studies are the most common [18, 19]. *Catharanthus roseus* contains significant amounts of volatile and phenolic compounds including caffeoylquinic acids and flavonol glycosides that are known to increase antioxidant activity. It has an important role in the body-defense system that acts as antioxidants against reactive oxygen species (ROS), which are dangerous, by developing such products through normal cell aerobic respiration. Accumulation of free radicals can cause pathological conditions such as ischemia, asthma, arthritis, inflammation, neurodegeneration, Parkinson's disease, mongolism, speeding up the aging process, and perhaps dementia. The flower petals, seeds and other parts of *Catharanthus roseus* exhibit antioxidant properties [20].

2. METHODS

2.1. Chemicals

These are some chemicals used for analysis in this research such as choline chloride, glycerol, rutin, quercetin, gallic acid, acetonitrile, and methanol that were produced by Sigma-Aldrich USA.

2.2. Deep Eutectic Solvents Preparation

According to the Peng *et al.* [21] DESs were prepared easily by the heating method, in which hydrogen-bond donors mixed with hydrogen-bond acceptors in the reaction flask for 60 min at 80°C under vigorous agitation to get the homogeneous liquid. In this research choline chloride and glycerol at 1:2 ratio were mixed in the in a reaction flask at 80°C for 60 min on a magnetic stirrer. Figure 2 shows the preparation of the DES.

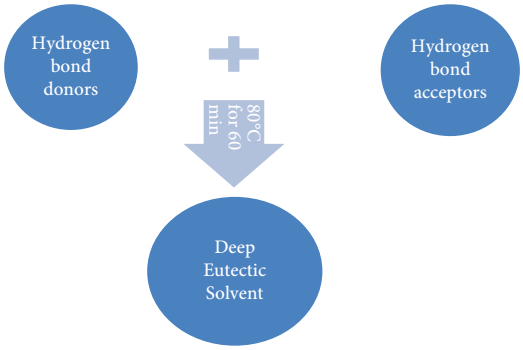
2.3. Extraction of Plant

In this study 10 g of dried plant parts (root, stem, leaves, and flower petal) powder of *Catharanthus roseus* was added into a flask with 150 ml of a DES solution. The flask was placed onto a magnetic stirrer at 50°C for 40 min. After that, the solution was filtered with the help of filter paper. Furthermore, the filtered solution was concentrated using a rotary evaporator; then the concentrated extract was collected and kept in a refrigerator at 4°C until the time of the experiment.

2.4. Isolation of Flavonoids from *Catharanthus roseus* Using HPLC

Samples were analyzed by using LC-20A HPLC system consisting of two pumps and UV detector set at 254 nm having a column size 250 mm × 4.6 mm (5 μ) hypersil gold-C18 (Thermo electron corporation). The combination of acetonitrile, methanol, and

Figure 2: Deep eutectic solvent preparations.



3% formic acid (50/ 50 /0.3, v/v/v) has been used as a mobile phase. The injection volume was 10 μl and the flow rate was set at 0.5 ml/min.

2.4.1. Preparation of Standard and Sample Solutions

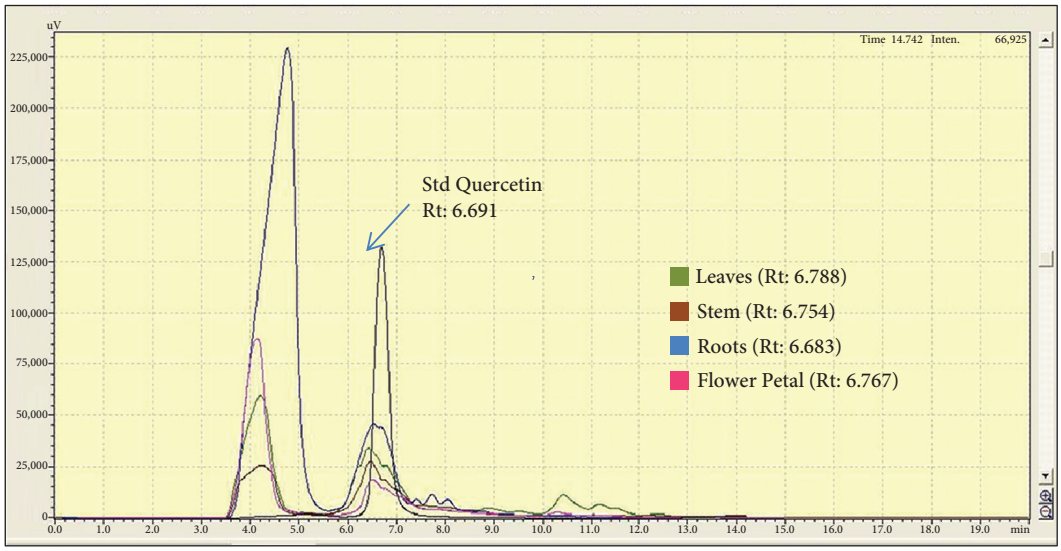
The combination of acetonitrile, methanol, and 3% formic acid (50/ 50 /0.3, v/v/v) has been used as a mobile phase and for the preparation of standard and sample, the mobile phase was used as a solvent; 10 mg of standard rutin, gallic acid, and quercetin were dissolved in 25 ml of the solvent (mobile phase). For sample preparation 10 mg of each extract of *Catharanthus roseus* plant parts (root, stem, leaves and flower petal) were dissolved in 10 ml of mobile phase as above as a standard preparation. The amounts of rutin, gallic acid, and quercetin in *Catharanthus roseus* parts were calculated by using the formula given below.

$$\%\ \text{Assay} = \frac{\textit{Sample Area}}{\textit{Standard Area}} \times \frac{\textit{Standard Weight}}{\textit{Standard Dilution}} \times \frac{\textit{Sample Dilution}}{\textit{Sample Weight}} \times \textit{Standard Purity}$$

3. RESULTS AND DISCUSSION

The identification of antioxidant compounds such as flavonoids, the secondary metabolites, are carried out because they are an important class of phytochemicals. Therefore, *Catharanthus roseus* parts were analyzed for the possible presence of these flavonoids. For this purpose, quercetin, rutin, and gallic acid were selected. The standard retention times (Rt) rutin, gallic acid, and quercetin were found to be 4.239, 6.388, and 6.691. The presence of rutin, gallic acid, and quercetin in *Catharanthus roseus* plant parts (root, stem, leaves, and flower petal) extract were found matching according to the standard retention time. Figures 3–5 illustrate the comparison between standard and *Catharanthus roseus* plant parts (root, stem, leaves and flower petal).

Figure 3: Comparison between Standard Quercetin and *Catharanthus roseus* parts.



In flower petal, rutin was found to be the retention time at 4.142, quercetin was found to have the retention time at 6.767. But the gallic acid got merged with another peak; that's why a separate peak is not seen. So, it is difficult to quantify gallic acid.

The *Catharanthus roseus* leaves showed the retention time of rutin was 4.206; gallic acid was found to be at 6.429 retention time, and quercetin was found to be at 6.788 retention time. In this chromatogram, the peaks of gallic acid and quercetin were merged little bit, but the retention time showed the presence of gallic acid and quercetin. Mardani *et al.* [8] have done work on the *Catharanthus roseus* parts (hairy roots, root, leaves, and callus) in methanol extraction, and their result showed the presence of gallic acid in all parts. It means that gallic acid is present in the leaves.

Gallic acid,rutin, and quercetin were also present in the stem at the retention time of 4.223, 6.465, and 6.754, respectively, and in the root quercetin was found to be at 6.683, but the rutin and gallic acid peaks merged with other peaks. According to the Mardani *et al.* in 2016 [22] gallic acid is present in root, so it indicates that the peak at the retention time of 6.524 may be gallic acid. Rutin in the root is not identified. The peak observed at the retention time (4.759) may have the rutin, but no separate peak has been seen, so may be the percentage is very less to identify.

The amounts of rutin, gallic acid, and quercetin were calculated using assay formula. In *Catharanthus roseus* flower petal the amounts of rutin and quercetin are 29.46 and 6.51%, respectively, whereas, rutin, gallic acid, and quercetin amounts

Figure 4: Comparison between standard gallic acid and *Catharanthus roseus* parts.

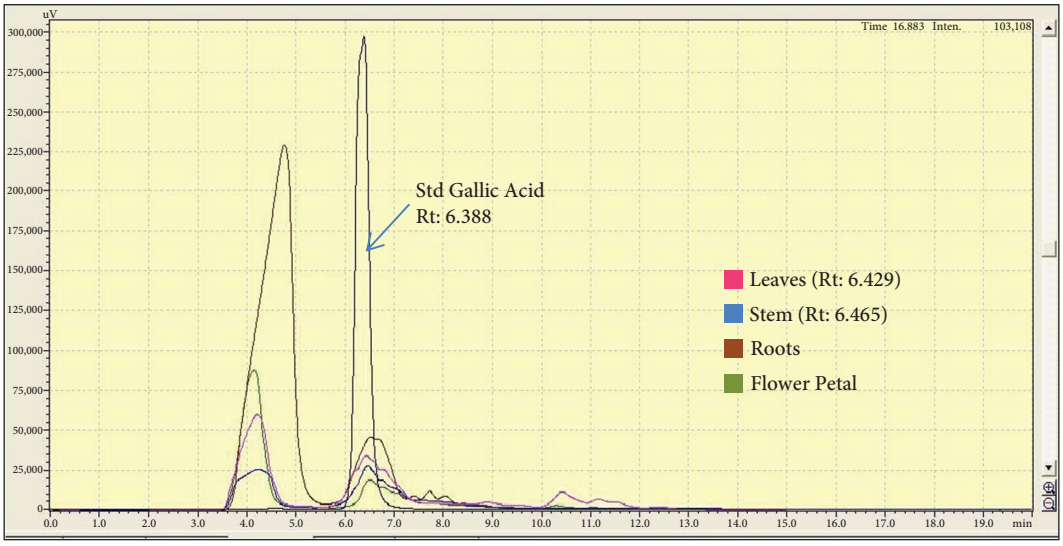


Figure 5: Comparison between standard rutin and *Catharanthus roseus* parts.

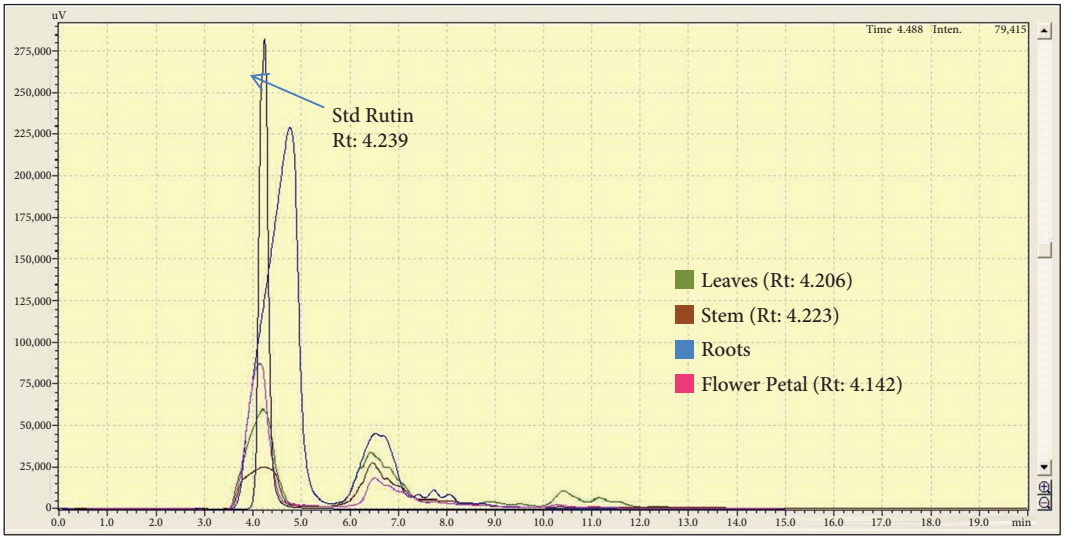
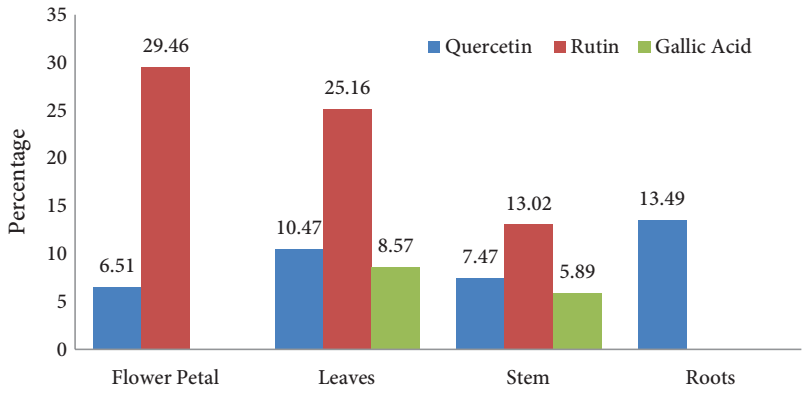


Figure 6: Amounts of quercetin, rutin, and gallic acid in flower petal, leaves, stem, and roots from HPLC analysis.



in leaves are 25.16, 8.57, and 10.47%, respectively, in stem, the amounts of rutin, gallic acid, and quercetin are 13.02, 5.89, and 7.47%, respectively. In root, only quercetin has been obtained that is 13.49%.

According to these results, the amount of rutin is higher in flower petal than leaves and lower in stem, and rutin is not identified in the root. The amount of gallic acid is higher in leaves than stem and not identified in flower petal and root. The higher value of quercetin in the root, a bit lower in leaves than in the stem, and the lowest amount has been observed in the flower petal. Furthermore, the graphical representation of these results is given below in Figure 6.

4. CONCLUSIONS

Catharanthus roseus has numerous effective invaluable therapeutic properties like anticancer, antidiabetic, antimicrobial, antioxidant, antihelminthic, antiulcer, antihypertensive, and antidiarrheal uses [23]. Nowadays, interests have been increasing in DESs based on choline chloride and their effective uses in extraction and separation of compounds from medicinal plants. Flavonoids [24, 25], aromatics [26], chalcones [27], and saponins [28] are the successful compounds being extracted and separated by using DESs from medicinal plants, and DESs extraction provide more efficient extraction than other solvents.

The isolation of rutin, gallic acid, and quercetin in DES extraction gave favorable results, and this is due to the formation of hydrogen bonds between flavonoids (rutin, gallic acid, and quercetin) and DES components. The HPLC is an analytical method, which was found to be an excellent technique for determination of rutin, gallic acid, and quercetin using DES extraction from plant parts of *Catharanthus roseus.* Hence this method can be applied for the qualitative and as well as quantitative analysis of rutin, gallic acid, and quercetin. Furthermore, the method was found to be simple, rapid and efficient. In *Catharanthus roseus* flower petal the amounts of rutin and quercetin are 29.46 and 6.51%, respectively, whereas, rutin, gallic acid, and quercetin amounts in leaves are 25.16, 8.57, and 10.47%, respectively. In stem the amounts of rutin, gallic acid and quercetin are 13.02, 5.89, and 7.47%, respectively. In root, only quercetin has been obtained that is 13.49%.

Author Contributions

All authors contributed equally to this study.

Source of Funding

None.

Conflict of Interest

None.

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