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# Determination of Phenolics and Flavonoids in Ethanolic Extract of Rosa Centifolia Using UV-Visible Spectroscopy

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**Abstract**

**Objective:** Our study aimed to analyze the phenolic properties of phytochemicals in ethanolic rose extract (Rosa centifolia L.). UV-Visible spectroscopic methods were used to achieve this goal. UV-Vis spectra helped identify the polyphenols present in the tested extract compared with the literature data. A large correlation was found between the antioxidant potential of the plant material (rose petals) tested.

**Materials and Methods:** The extraction process itself, as well as the processing of rose petals, has undoubtedly significantly reduced the antioxidants present in it. Rose petals were extracted by distillation method using a soxhlet apparatus for 4 hours and fractionated by using various solvent systems, petroleum ether: chloroform, chloroform: ethyl acetate, and ethyl acetate: ethanol of increasing polarity (100:0 to 0:100 with constant polarity increase of 10%) 10 fractions of each solvent system.

**Results:** The ethanolic extract UV-visible spectroscopy profile showed peaks at 268, 270, 285, 401, and 408 nm with the absorption 0.0128, 0.0126, 0.0051, 0.162, and 0.142 respectively. The final fraction of the extract shows 255, 257, 260, 273, 307, 401, and 408 nm with the absorption 0.0190, 0.017, 0.0399, 0.0279, 0.0181, 0.354, and 0.250 respectively.

**Conclusion:** The analysis of the sample shows a noticeable presence of polyphenolic compounds Rose petal extracts can be an alternative to synthetic compounds in cosmetics, food, or medications, such as Trolox, BHA, and BHT.

**Keywords:** UV- visible spectroscopy; Rosa centifolia; Polyphenolics; Quercetin

## 1. Introduction

Herbal medicines have established an important part of traditional medicine from ancient times. Modern science has documented the active component of plant origin, and a variety of drugs now it has been involved in the modern way of pharmacotherapy, which is identified by ancient cultures and used all years ago the times (1). Phenolic compounds are bioactive molecules. Furthermore, phenolic compounds have been reported as effective antioxidants. This phenolic group of compounds is at the attention of significant research. Phenolic compounds having various preventive roles against inflammatory, neurodegenerative, cardiovascular diseases, or anticancer activity have been widely acknowledged. The phenolic compounds are generally classified into two main categories non-flavonoids and flavonoids (2,3). Moreover, flavonoids come under the polyphenols category that contains at least two phenolic rings and they are further categorized into different sub-class such as flavonols, flavonones, flavones, flavanolols, flavan-3-o1s, and isoflavones. The phenolic and flavonoids compounds antioxidant activity is directly correlated with the presence of the sample's hydroxyl (-OH) group. Further, the positions of hydroxyl groups also decide the

generation of free radical scavenging activity. The phenolic compounds have already been proven to have many therapeutic effects such as antimicrobial, antioxidant, anticancer, and antidiabetic (4,5)**.** UV-visible spectroscopy has been confirmed to be one of the most appropriate and reliable techniques to quantify these phenolic compounds. UV and visible light are widely used for the quantification of chemical compounds (6). UV spectroscopy, also known as ultraviolet-visible, refers to absorption or reflectance spectroscopy in the ultraviolet-visible region. This means that UV spectroscopy uses adjacent ranges of visible light and visible light. The perceived color of the chemicals involved directly affects the absorption or reflectance in the visible range. The atoms and molecules undergo electronic transitions under the electromagnetic spectrum region. Absorption spectroscopy absorption measures the transition from the ground state to the excited state. complementary to fluorescence spectroscopy, in that fluorescence deals with transitions from the excited state to the ground state (7,8). Due to their biochemical properties and molecular arrangement, Phenolic compounds are highly appropriate for identifying and quantifying with UV-visible light. The ability to absorb UV light by the phenolic ring present in the structure is exploited to quantify such compounds. Among these, the use of UV-visible spectrophotometry to approximate the content of phenolic compounds makes it the most widely used technique. UV-VIS spectroscopic is an easy, cost-effective, and quick test for detecting phytochemicals (9, 10). In this manuscript, we discussed UV-visible analytical techniques available for the quantification of phenolic content in the ethanolic extract of rose and its final fraction. Moreover, the UV-visible spectral features observed in crude extract and in the final fraction are also reported and discussed.

## 2. Materials and Methods

### 2.1Chemicals

All chemicals used were of analytical grade (Sigma-Aldrich) and supplied by Pandit Ravishankar Shukla University, Raipur (Chhattisgarh). The experiments were performed in non-aqueous media.

### 2.2 Collection and authentication of plant materials

*Rosa centifolia* flowers were collected from the botanical garden Koni Bilaspur C.G. The samples were properly packed in polyethene bags to avoid the decomposition of phytochemicals. Sample identification and authentication were performed by Professor A. K. Dixit, Department of Botany. This identified sample was used as reference material.

### 2.3 Preparation of extract and fractionation

The fresh flower petal was cut into minute pieces 200 gm samples were extracted by using a soxhlet apparatus for 4 hours, ethanol was used in this extraction process. After complete extraction, the solvent present in the filtrate was evaporated using a rotary evaporator under a vacuum, and after 30-35 min semisolid crude extract was weighted approximately 5.17 gm [19,20]. The crude extract of *Rosa centifolia* is separated into various components by column chromatography technique. The ethanolic crude extract was weighed 3.75 gm and fractionated by using silica gel (60–120 mesh), and the column was used (85 cm × 5.7 cm). Following bioassay-guided fractionation protocol with slight modification in the procedure [21, 22]. Elution was performed with different proportions of petroleum ether: chloroform of increasing polarity (100:0 to 0:100 with constant polarity increase of 10%) to give 10 fractions (F1 to F10), monitored by preliminary study and using TLC plates and the fractions F5 and F6 with similar Rf values in TLC pattern were pooled together. Eluted with chloroform: ethylacetate (100:0 and 0:100). Further column purification of F6 (with greater similarity as compared to F5) yielded fractions denoted F6.1 to F6.10. Fractions F6.4 were further applied to chromatographic silica gel columns (27.5 × 2 cm) and eluted with ethyl acetate: ethanol (100:0 and 0:100, 500 ml each fraction). Fraction F6.4 afforded ten sub-fractions (F6.4.1 to F6.4.10) of which F6.4.2, TLC pattern of this fraction represented similar Rf value and it is collected as a final fraction for the preparation of sample stock solution [23, 24].

### 2.4 Instrumentation and spectroscopic condition

The UV/Vis spectra were recorded with a spectrophotometer (UV-1650PC, Shimadzu, Kyoto, Japan). Quartz cells (1cm) were used for the measurement of all absorbance. The spectra for all scans from range 200 to 800 nm. Samples were predisposed to plastic cuvettes supplied by Eppendorf (Hamburg, Germany) and

covered by cardboard to shield the cuvette from stray light. The spectra were normalized by setting the absorbance at 800 nm equal to zero. The standard procedure was applied for all the samples. The data matrix was processed by FITOPAC v 2.1.2 software and the data was automatically reduced to an ASCII file.

**2.5 Preparation of standard**

To examine the absorption characteristics of the standard compound. Prepare a dilute solution of the standards by dissolving 1 mg in 10 mL of 96% ethanol and filtering through the Whatman No. 1 filter paper. The standard solution of quercetin was scanned separately using a spectrophotometer.

**2.6 Preparation of sample**

To examine the absorption characteristic of extract visible and UV light. For UV-VIS spectrophotometer analysis, the extract was sonicated for 10 min and filtered through Whatman No. 1 filter paper. The sample is diluted to 1:10 with the same solvent 96% ethanol. Ethanol was considered a blank solution.

**3. Result**

**3.1 UV-Visible Spectroscopy**

The literature shows that most flavonoids have absorption bands in the range of 240 to 280 nm and 300 to 400 nm (12, 13). This range of wavelengths of the absorption bands strictly follows the structure of the polyphenol molecule, including the conjugation degree, the number and position of substituents, and the OH groups (14, 15). Figure 1 and 2 shows the UV-VIS spectrum of the rose extract. In the 300-450 nm range, we noticed two maxima of absorption as a shoulder, which corresponds to different phytochemicals. The spectral characteristics (UV) of flavonols, which are present in the extract studied, give the absorption bands 200-800 nm. The qualitative UV-VIS profile of the ethanolic extract of Rosa centifolia (sample) was taken at the wavelength of 200 nm to 800 nm due to the sharpness of the peaks and proper baseline. The profile showed the peaks at 268, 270, 285, 401, and 408 nm with the absorption 0.0128, 0.0126, 0.0051, 0.162, and 0.142 respectively. The final fraction of the extract shows 255, 257, 260, 273, 307, 401, and 408 nm with the absorption 0.0190, 0.017, 0.0399, 0.0279, 0.0181, 0.354, and 0.250 respectively. Fig. 1 (a) and (b) show the absorption spectrum of Rosa centifolia extract and its fractions. Absorption bands were observed in both samples of Rosa centifolia plant extract and its fraction is represented in Tables 1 and 2.

**Table 1:** UV-VIS peak values of extract of Rosa centifolia.

| S. No. | Wavelength (nm) | Absorption |
|---|---|---|
| 1. | 268 | 0.0128 |
| 2. | 270 | 0.0126 |
| 3. | 285 | 0.0051 |
| 4. | 401 | 0.162 |
| 5. | 408 | 0.142 |

**Table 2:** UV-VIS peak values of extract of Rosa centifolia final fraction.

| S. No. | Wavelength (nm) | Absorption |
|---|---|---|
| 1. | 255 | 0.019 |
| 2. | 257 | 0.017 |
| 3. | 260 | 0.0399 |
| 4. | 273 | 0.0279 |
| 5. | 307 | 0.0181 |
| 6. | 401 | 0.354 |
| 7. | 408 | 0.250 |

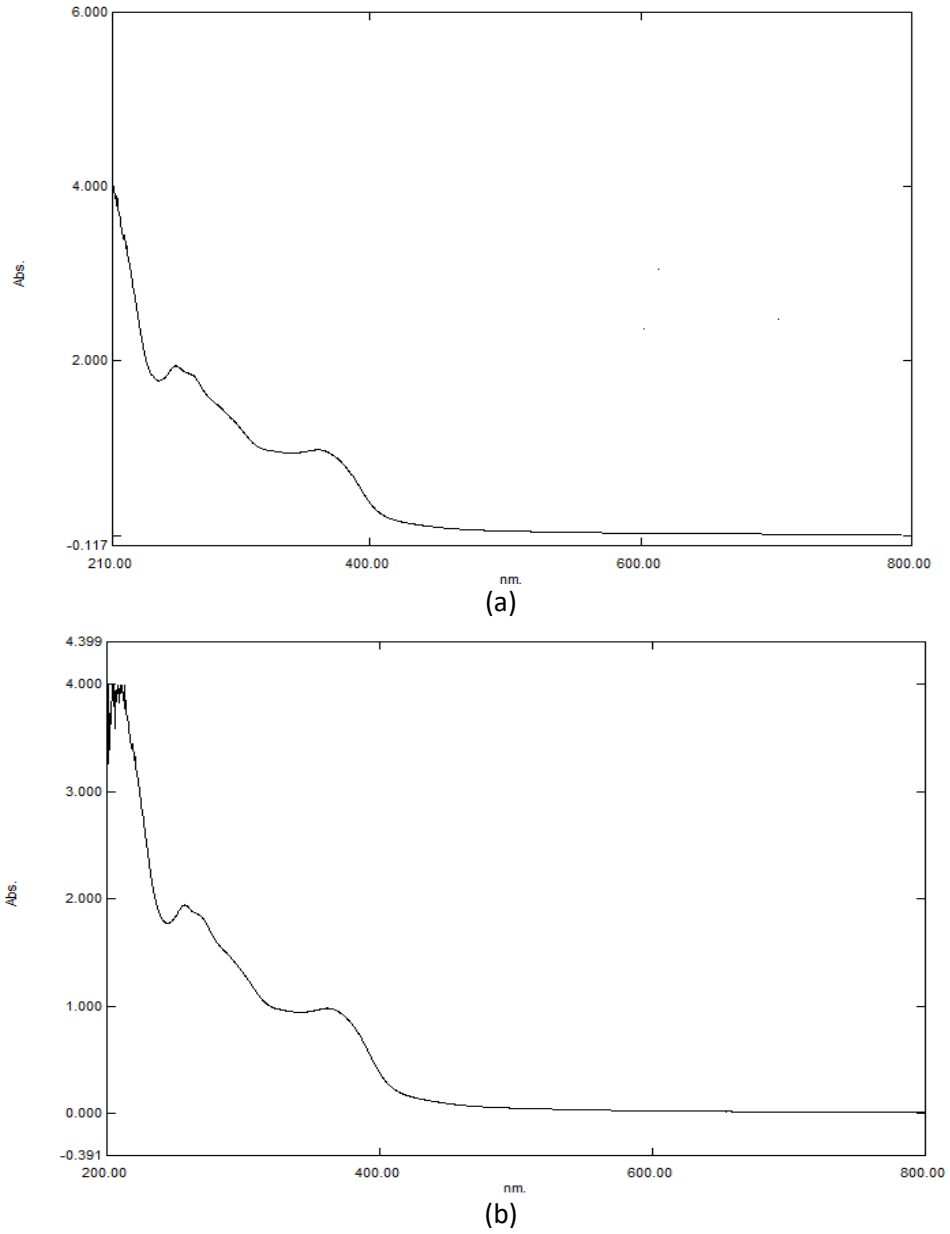


**Fig. 1:** Figure 1: UV-VIS spectra of (a) pure ethanolic Rosa centifolia extract (b) final fraction of extract.

## 4. Discussion

Our investigation is the study of the UV-VIS analysis for the identification of phytochemicals present in the ethanolic extract of *Rosa centifolia*. The UV-visible spectra were helpful in the identification of σ-bonds, π-bonds, and lone pairs of electrons, chromophores, and aromatic rings present in the compound. The presence of unsaturated groups and heteroatoms such as S, N, and O is indicated by the appearance of one or more peaks in the region from 200 to 400 nm in the UV-VIS spectra (16). Nevertheless, the application of UV-visible spectrophotometry is limited for complex media. Analysts have difficulties in characterizing absorption peaks to any certain constituents in the extract. Thus, UV-VIS findings must be accompanied by some other analytical technique such as HPLC, GC/MS, etc., to properly characterize and identify the constituent of the extract (17, 18). The qualitative UV-VIS profile of the ethanolic extract of *Rosa centifolia* was applied at the wavelength of 200 nm to 800 nm due to the sharpness of the peaks and proper baseline observation. The UV-spectra profile showed that the sample (ethanolic extract and its final fraction) absorbance peaks in the range of polyphenolic compounds. Figure 1 (a) and (b) show the absorption spectrum for both. Absorption at different wavelength bands observed pertaining to the ethanolic extract of *Rosa centifolia* patel is displayed in Table 1. Figure 1 shows the UV-VIS spectral band of the rose petal extract. In the 250-410 nm range, we noticed two maxima of absorption as a shoulder, which resembles

different phytochemicals. The spectral characteristics (UV) of flavonols, which are present in the extract studied, give absorption bands in the range of 270-285 nm (14). Quercetin gives an absorption band at 268, 270, and 374 nm, Chrysin, hesperetin, eriodictyol, and taxifolin give an absorption band at 288 nm(19), (+) catechin at 280 nm, and (-) epicatechin at 278 nm (20,21). UV-VIS spectroscopic analysis of the extract showed the presence of phenolic and flavonoid compounds, indicating high levels of organic acids (gallic, cinnamic, and ellagic), polyphenolic compounds, and flavonoids (rutin, kaempferol, quercetin, and catechin) which can be isolated for further studies for various types of pharmacological activities.

## 5. Conclusion

In the present study analysis of the petal extract of *Rosa centifolia* under UV-VIS spectroscopy, the analysis of the sample shows a noticeable presence of phenolic and flavonoid compounds which can be isolated and screened for further various types of medicinal efficacy depending on their clinical uses.

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