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Research Article

Physiochemical Evaluation and Determination of Chemical Constituent in Rose Petal (*Rosa centifolia*)

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Abstract

Objectives: The purpose of the present study was to explore the Pharmacognostic parameters for the standardization of *Rosa centifolia* Petals.

Material and Methods: The flowers of *Rosa centifolia* were authenticated and shade dried. *Rosa centifolia* petals were collected then macromorphological, physiochemical assessment, and the micrometric study was carried out. The dried powder form of the *Rosa centifolia* Petals was then extracted with different solvent systems alcoholic, ethyl acetate, and petroleum ether, and their extractive values were calculated. Most of the phytochemicals were found in ethanol and ethyl acetate fractions. Thin Layer chromatography (TLC) of the ethanol, ethyl acetate, and petroleum ether extract was performed for important phytochemicals flavonoids and polyphenol. Flavonoids and phenolics showed their presence in all extracts with one spot in each extract for ethanol, ethyl acetate, and petroleum ether.

Result: The Physicochemical exploration showed values for moisture content, moisture sorption capacity, ash values, and extractive values which are within the limits of World Health Organization standards for the crude drug from medicinal plants. Micromeritic analysis of petal powder reveals good flowability. Ethanolic extractive values were found to be higher when compared to extractive values of, ethyl acetate and petroleum ether. Preliminary Phytochemical examination for the sample indicated the presence of carbohydrates, glycosides, alkaloids, flavonoids, and amino acids. Rf value for flavonoid and phenolic on TLC were found to be for ethanol 0.78 and 0.77, for ethyl acetate 0.81 and 0.78, for petroleum ether 0.81 and 0.78 respectively.

Conclusions: The current research would be useful to supplement the information regarding pharmacognostical characteristics, physicochemical evaluation, micrometric analysis, and phytochemical exploration in the Ayurvedic system of medicine for its identification and medicinal use.

Keywords: *Rosa centifolia*, Macromorphological description, Physicochemical evaluation, Phytochemical screening, TLC screening.

INTRODUCTION

The worldwide occurrence of diabetes mellitus is rising in adults constitutes global public health trouble. It is predicted that India, China, and the United States will have the largest number of people with diabetes by 2030. By explanation, diabetes mellitus is categorized under metabolic disease and characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The huge mainstream of cases of diabetes falls into two etiopathogenetic categories. In one category, type 1 diabetes, the cause is a complete deficiency of insulin secretion. In the other, a much more common category, type 2 diabetes the cause is a combination of resistance to insulin action, and an insufficient compensatory insulin-secretary response[1]. The key components of type II diabetes are β-cell dysfunction causing impaired insulin secretion and increased need for insulin due to insulin resistance[2]. Metabolic changes caused by hyperglycemia are called diabetes mellitus and hyperglycemia. The most common hypoglycemic agents used globally such as metformin, sulfonylureas, and glucosidase inhibitors have severe adverse effects such as diabetic ketoacidosis, diarrhea, and a variety of diabetes complications. The unbeaten treatment and management of diabetes are yet to be exposed. Within the Indian subcontinent, extensive research has been performed in the ethnomedicine system to find out the possible uses of the plant as an anti-diabetic agent[3]. Animal models of diabetes are therefore greatly useful and advantageous in biomedical studies because they offer a promise of new insights into human diabetes. Most of the available models are based on rodents because of their small size, short generation interval, easy availability, and economic considerations[4]. Abundant studies have been also published on the antioxidant, antibacterial activities, and chemical composition of rose essential oil, in current years. However, no report on the antioxidant and anti-diabetic activities and total phenolic contents of Rosa centifolia are available to

date, for our information. Rose essential oil use in the perfumery and cosmetic industry, it has high market value because of its. Hydrosol and absolute are less expensive In comparison with rose oil. Consequently, the purpose of the present study was to investigate the antidiabetic properties of rose essential oil, which is used as raw material in various cosmetic and pharmaceutical applications[5].

MATERIAL AND METHOD

Collection of Plant sample

The petals of *Rosa centifolia* sample were collected from botanical garden Koni Bilaspur C.G. The samples were packed instantly in polyethylene bags to avoid decomposition of some bioactive compounds Botanical identification was performed by Professor A. K. Dixit, Department of Botany.

Macromorphological description of *Rosa centifolia* petals

The *Rosa centifolia* plant part petals were subjected to macroscopic studies along with the organoleptic characteristics such as color, odor, taste, texture, shape, and size of the drug.

Physiochemical evaluation of *Rosa centifolia* petals

Physiochemical parameters for *Rosa centifolia* plant part petals such as moisture content, ash constants, pH, and soluble extractive values of *Rosa centifolia* petals were performed according to the official method prescribed and the WHO guidelines on quality control methods for medical plants Material[6].

Micromeritic evalution of *Rosa centifolia petals*

The micrometric characteristics for powder form of *Rosa centifolia* petals such as Bulk density, Tapped Density, The angle of repose, Hausner's ratio, and Carr's index were determined according to the official standard procedure [6].

Preparation of ethanol extracts and fractions

The minute pieces of fresh flower petal samples (200 gm) Ethanol as solvent were selected for extraction of plant material. Sample powders of the plant were soaked in ethanol (5.0 L) in a percolator. The percolation process was repeated up to three to five times till the plant material was extracted completely. After complete extraction, the sample was filtered with filter paper and the solvent was evaporated using a rotary evaporator under pressure at 40-45 C for 30 min resulting in a semisolid crude extract and weighed (5.37 g). A small quantity (0.37 g) of Crude ethanol extract was transferred in a test tube for another study. The crude ethanol extract was dissolved in distilled water (500 ml) and then successively partitioned (5 times each) with petroleum ether and ethyl acetate in a separating funnel. After extraction, all crude extracts were put inside the fume hood for few days. After the solvent evaporates, the ethyl acetate (1.30 g) and petroleum ether crude extracts (1.62 g) were obtained[7].

Preliminary phytochemical study

The stock solution was prepared from the ethanol extract, ethyl acetate, and petroleum ether fractions of *Rosa centifolia* extract. The obtained stock solutions were subjected to preliminary phytochemical screening for the identification of chemical constituents.

Test for carbohydrates

Molisch's test for carbohydrate

Approximately 500 mg of crude extracts were dissolved in 5 mL of distilled water each and later filtered. A few ethanol were added to these filtrates. Then 1 mL of concentrated H_2SO_4 was poured carefully along the side of the test tube. After two minutes, 5 mL of distilled water was added. A positive test, indicating the presence of carbohydrates, was confirmed with the formation of dull violet or red color at the interphase of the two layers[8].

Fehling's test for reducing sugar

Crude extracts (2.5 mg) were dissolved individually in 1.5 mL of distilled water and filtered. Next, Fehling's solutions A and B (a ratio of 1:1) were added to the filtrates about 1 mL mixture, then heated on a water bath for 5 minutes. The brick-red precipitate confirmed the presence of reducing sugars[8].

Test or starch

In 2.5-3.5ml of the extract and 0.01 gm of Iodine and 0.075 gm of KI were dissolved in 5ml of distilled water were added. The creation of a blue color indicated the presence of starch[9]

Tests for alkaloids

Mayer's test

Mayer's reagent (potassium mercuric iodide solution) giving a cream color precipitate.

Wagner's test

Wagner's reagent (iodine-potassium iodide solution) yielding reddish-brown precipitate.

Dragendorff's test

Dragendroff's reagent (potassium bismuth iodide solution)[10]

Test for tannins

FeCl3 test for tannins

Dissolved in 10 ml distilled water and then filtered. A few drops of 1% Iron chloride (FeCl3) solution were added. Black or blue-green coloration or precipitate indicates the positive result for the presence of tannins in the test samples[11]

Test for flavonoids

Sulphuric acid test

On the addition of sulphuric acid (60-80%), flavones dissolve into it and give a deep yellow solution. Flavones give an orange to reddish color[11]

Test for steroid

Liebermann-Burchard reaction

Mix 2 ml extract with chloroform. Add 1-2 mL acetic anhydride and 2 drop conc. Sulphuric acid from the side of the test tube. The color appears first red, then blue, and finally green[11]

Test for amino acid and protein

Ninhydrin test

The ninhydrin test uses to identify the presence of alpha-amino acid and protein-containing free amino groups. The crude extract (sample solution) when heated with ninhydrin molecules, gives characteristics of deep blue or pale yellow

due to the formation of a complex between to ninhydrin molecule and the nitrogen of free amino acid[12]

Test for glycosides

Anthraquinone glycoside (Borntrager's test)

To the crude extract solution (1 mL), 5% H2SO4 (1 mL) was added. The mixture was boiled and then filtered. The filtrate was then shaken with an equal volume of chloroform and kept standing for a few minutes (5min). Then a lower layer of chloroform was shaken with half of its volume with dilute ammonia. The formation of rose-pink to red color of the ammonical layer indicates anthraquinone glycoside[13].

Cardiac glycoside (Keller-killiani test)

The crude extract (1 g) was shaken with distilled water (10 mL). To this, glacial acetic acid (4mL) containing a few drops of ferric chloride was added, followed by H2SO4 (2 mL) along the side of the test tube. A positive indication for cardiac glycoside is the formation of a brown ring at the interface and a violet ring may appear just below the brown ring[13].

Total phenolic content

The amount of total phenolic content (TPC) by Folin-Ciocalteu reagent, the method described by Singleton and Rossi was used. Briefly, 0.5 mL of three different extracts were mixed with and 0.5 mL of distilled water and added to 2.5 mL of diluted Folin's reagent, followed by the addition of 2 mL of 7.5% (w/v) sodium carbonate. After incubation in the dark, their absorbance was determined by spectrophotometer at 750 nm. All measurements were repeated three times and the concentration of total phenolic content was expressed as µg of gallic acid equivalent per gram of the extract[14].

Total flavonoid content

With minor modification, the method of Sawsan Rahimuddin *et al.* (2013) was used to determine the total flavonoid content of rose petals extract. 250 µl of the three different fractions of crude extracts (sample solution) was added to 1.25 ml deionized water and 75 µl of 5% NaNO2 (w/v). After 5 min, 150 µl of 10% AlCl3 and 0.5 ml of 1 M NaOH (w/v) were added. The volume was completed to 2.5 ml with deionized water and measured at 510 nm. All measurements were repeated three times the concentration of total flavonoid content was expressed as µg of quaricitine equivalent per gram of extracts[15].

Thin-layer chromatography profiling of extract

The TLC profiling was performed as described by Biradar *et al.*, 2013. The TLC plates were prepared by using Silica gel 'G' as 20 gm of silica gel was weighed and made to a homogenous suspension with 50 ml distilled water for two minutes, this suspension was distributed over the plate which was air-dried until the transparency of the layer disappeared. The plates were dried in a hot air oven at 110°C for 30 min and then stored in a dry atmosphere and used whenever required. Samples were prepared by diluting the crude extracts of petroleum ether, ethyl acetate, and ethanol with respective solvent and then applied usually 1-10µl volumes to the origins of a TLC plate 2cm above its bottom with the help of capillary tubes[16].

Rf= Distance traveled by the streak from the starting point / Distance traveled by the solvent from the starting point to the solvent front.

The fractions were kept at 4^0C in the refrigerator for further work.

Developing system

The TLC studies were performed for the detection of flavonoid and phenolic compounds using the solvent systems petroleum ether, ethyl acetate, and ethanol (7: 2: 1). The plates were air-dried and visualized by the presence of one spot on the TLC plate was in the I2 vapor and UV lamp. In I2 vapor spot appears green color while in UV lamp spot gives greenish-brown color. The relative front (Rf) of each fraction was calculated. Spots were separated by running on preparative TLC plates in the selected solvent systems and collected by scrapping silica from the TLC plate for further study. [17,18]

RESULT AND DISCUSSION

Macromorphological description of *Rosa centifolia* petals

The Rosa centifolia flowers are moderate pink to light red with relatively small flowers grow in groups. In this study, the pharmacognostical data on the Rosa centifolia petals can assist as a relevant source of information and contribute towards the standards for its identification and authentication. The results of macromorphology were shown in table 1. The Fresh Petals, dried, and powdered Rosa centifolia flowers were illustrated in figure 1. According to the World Health Organization (WHO), medicinal plants would be the best source to obtain a variability of drugs and medicinally active compounds. Therefore, herbal plants should be investigated to understand their properties and efficiency[19].

The consistency and extractive values of different extracts of *Rosa centifolia* petals

An extractive value signifies the number of constituents present in the given amount of plant material extracted with different solvents. To achieve a higher recovery of the required compounds due to the polarity and ability of water to interact with the verities of groups. Besides, the density of the fluid mixture may increase(20). It indicates the extent of polar, medium polar, and non-polar constituents present in the crude drug. In the present study, alcohol soluble extractive values were found to be more when compared to ethyl acetate and petroleum ether soluble extractive values, which denotes that *Rosa centifolia* petals contain more quantity of polar compounds.

Table 1: Macromorphological description of *Rosa centifolia* petal

S. No.	Characters	Observations
	Organoleptic Characters	
1.	Colour	Magenta on base and light yellow near to apex
2.	Odor	Aromatic Distinct
3.	Taste	Distinct tongue sensitizing aromatic taste with pleasant mild sweetness
	Quantitative Macromorphology of Fresh petals	
4.	Width	0.9-3.7cm
5.	Length	1.7-4.2cm
	Extra Features	
6.	Shape	Heart/ Pear Shape
7.	Texture	Soft and smooth

Fig.1: The Fresh Petals, Dried, and powdered *Rosa centifolia* flowers

Table 2: The consistency and extractive values of different extract of *Rosa centifolia* petals

S. No.	Treatment	Consistency	Extractive values (%)
1.	Ethanol	Sticky	17.27
2.	Ethyl acetate	Sticky	13.23
3.	Petroleum ether	Sticky	6.98

Physicochemical parameters of *Rosa centifolia* petals

Loss drying is used to estimate the amount of volatile matter including water that is present in the plant material. Percent Loss on drying was found to be 14.30%. The moisture content of a drug should be reduced to avoid decomposition of crude drugs, either due to chemical change or microbial contamination. The percentage of moisture content ranging from 10 - 20% indicates an ideal range for bacteria as well as for fungal growth. The extent of polysaccharide that is present in certain drugs is represented by the swelling index. If the swelling index of powder changes it indicates that the powder has been not properly stored it is one of the characteristics for the identification of botanical drugs. For the *Rosa centifolia* drug, the swelling factor in water after 24 hours was found to be 4.8 ml. The ability of a drug to take up water is determined by the drug. Acid-insoluble ash indicates the presence of only earthy matter i.e., sand or silica in the drug sample while Water-soluble ash detects the drug shattered with water if blend with the exhausted material will show a much greater reduction in water-soluble ash than total ash. So, when exhausted material is substituted for the genuine drug it's an important pointer. In the present study Total ash, Acid insoluble ash, and Water-soluble ash were found to be 6.35, 1.51, and 2.49 % w/w respectively.

Table 3: Physicochemical parameters of *Rosa centifolia* petals

S. No	Constants	Yield (N=3)
1.	Foreign matter	0
2.	Moisture content(Loss on drying) %	14.30 %
3.	Ph	6.56
4.	Swelling Index ml	4.8
5.	Moisture Sorption Capacity /g	0.72
6.	Total ash (% w/w)	6.35
7.	Acid insoluble ash (% w/w)	1.51
8.	Water soluble ash (% w/w)	2.49

Micrometric parameters of *Rosa centifolia* petals

The Micromeritic properties like Bulk density, Tapped Density, Angle of repose, Hausner's ratio, and Carr's index were determined as a part of the micrometric analysis. Carr's compressibility index and Hausner's ratio give the insight value of the difference in the bulk and tapped densities. While Carr's index shows strength, ability and predicts solubility of the crude drug. In preparation of drugs, this information are important(21). Hausner's ratio reveals inter particulate friction in between particles. As the values, these indices decrease the flow property of the powdered drug increases. The angle of repose is a traditional characterization method for determining the flow property of powder. The result showed that the powder has good followability as the angle of repose of powder was found to be 31.22.

Table 4: Micrometric parameters of *Rosa centifolia* petals

S. No	Constants	Yield
1.	Bulk density	0.203g/ml
2.	Tapped Density	0.258 g/ml
3.	Angle of repose	31.22
4.	Hausner's ratio	0.779
5.	Carr's index	22.22 %

Preliminary phytochemical screening of different extracts of *Rosa centifolia* petals

Phytochemical analysis of fractionated portions of the *Rosa centifolia* extract (ethanol, ethyl acetate, and petroleum ether) concluded that the plant petal extracts contain many secondary metabolites such as flavonoids, polyphenols, steroids, and terpenoids. Phytochemical screening of Rosa centifolia petals showed that maximum phytoconstituents are present in ethanolic and ethyl acetate extract. Medicinal plants contain the component of therapeutic value. Medicinal herbs have curative properties due to the presence of various complex chemical substances with a different compositions which are found as secondary metabolites in various parts of the plant. Secondary metabolites play a role in the medicinal properties of the plant in herbal plant flavonoids are another most active plant constituents with antibacterial and antifungal properties(22).

Table 5: Preliminary phytochemical screening of different extracts of *Rosa centifolia* petals

S. No	Plant constituent	Ethanolic Extract	Petroleum ether	Ethyl acetate
1.	Alkaloids	Positive	Negative	Positive
2.	Glycosides	Positive	Positive	Positive
3.	Carbohydrates	Positive	Negative	Negative
4.	Flavonoids	Positive	Positive	Positive
5.	Tannins	Positive	Positive	Positive
6.	Proteins	Positive	Negative	Positive
7.	Amino acids	Positive	Negative	Positive
8.	Sterols	Positive	Positive	Positive
9.	Starch	Positive	Negative	Negative
10.	Cardiac glycosides	Positive	Negative	Positive

Total phenolic content (TPC) and total flavonoid content (TFC)

Flavonoids and phenolics have attracted a great deal of attention due to their potential health benefits. Over the past few years, several experimental studies have demonstrated the biological and pharmacological properties of many flavonoids and phenolics, especially their antimicrobial activity, anti-inflammatory, antioxidant hypoglycemic, and anti-diabetic effects. Phenolic components such as phenolic acids, tannins flavonoids, etc. are considered the most substantial phytochemical components produced by plants. These compounds are existing in various parts of the plant and their quantities significantly depend on the kind of the plant organ, climate, variety, location, etc(23). The estimation of the TPC of plants became an important tool to understand the plant's antioxidant benefits. Table 6 shows the fresh rose petal content of phenolic compounds 388 ± 0.01 µ g/mg of ethanol fraction, 235 ± 0.01 µg/mg of ethyl acetate fraction, and 211 ± 0.01 g/mg of petroleum ether fraction. In distinction, the TFC was lesser 17.8 ± 1.1 µg/mg of ethanol fraction, 3.1 ± 0.05 µg/mg of ethyl acetate fraction and 1.8 ± 0.08 µg/mg of petroleum ether extract.

Table 6: Total phenolic and flavonoid content of *Rosa centifolia* fresh petal aqueous extract

Fractions (mg/ml)	Total phenolic content (µg/mg of gallic acid)	Total flavonoid content (µg/mg of Quaricitine)
Petroleum ether	211 ± 0.01	1.8 ± 0.08
Ethyl acetate	235 ± 0.02	3.1 ± 0.05
Ethanol	388 ± 0.01	17.8 ± 1.1

Comparative TLC observations of flavonoid and Phenolic compound

The presence of various phytochemicals strengthens by the TLC study revealed the presence of tannins, saponins, glycosides, and flavonoids which are compounds capable of causing varied physio-chemical and pharmacological effects. Their presence therefore plant showed potential for the development of drugs against many diseases and seems to support the traditional use of the unexplored plant *Rosa centifolia*. A similar separation pattern was observed in all fractions of extract shown in figures 2 and 3, results of TLC profiling are summarized in Table 7 and Table 8.

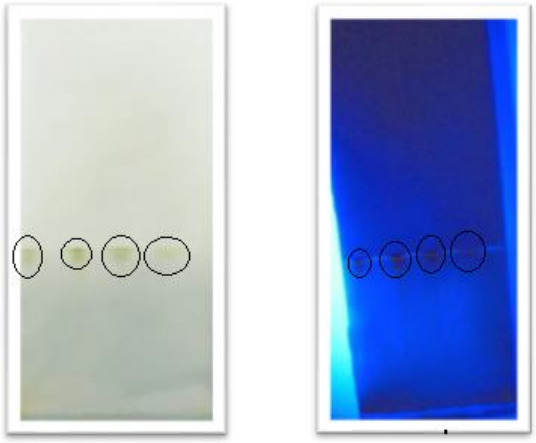

Figure 2: Comparative TLC observations of Phenolic compound from different fractions of *Rosa centifolia*.

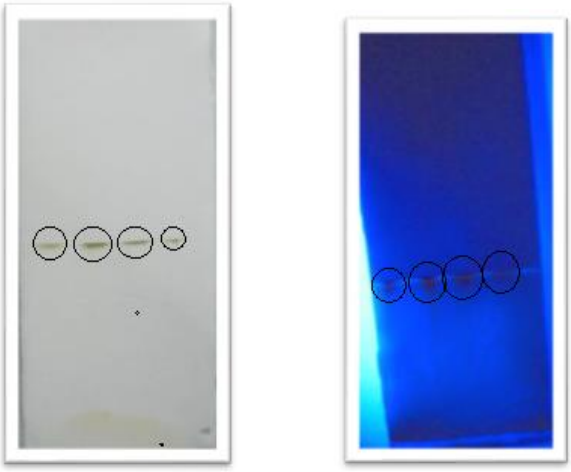

Figure 3: Comparative TLC observations of flavonoid compounds from different fractions of *Rosa centifolia*.

Table 7: Comparative TLC observations of phenolic compound from different fractions of *Rosa centifolia*.

S. no.	Fraction	Mobile phase Petroleum ether, ethyl acetate, and ethanol (7: 2: 1)								
		Eye			I_2 vapour			Uv lamp		
		Spot No.	Color	Rf	Spot No.	Color	Rf	Spot No.	Color	Rf
1	Petroleum ether	1	Green	0.77	1	Green	0.77	1	Orange	0.77
2	Ethyl acetate	1	Green	0.77	1	Green	0.78	1	Orange	0.78
3	Ethanol	1	Green	0.77	1	Green	0.77	1	Orange	0.77

Table 8: Comparative TLC observations of flavonoid compound from different fractions of *Rosa centifolia.*

S. no.	Fraction	Mobile phase Petroleum ether, ethyl acetate, and ethanol (7: 2: 1)								
		Eye			I_2 vapour			Uv lamp		
		Spot No.	Color	Rf	Spot No.	Color	Rf	Spot No.	Color	Rf
1	Petroleum ether	1	Yellowish green	0.78	1	Green	0.78	1	Orange	0.78
2	Ethyl acetate	1	Yellowish green	0.81	1	Green	0.81	1	Orange	0.81
3	Ethanol	1	Yellowish green	0.81	1	Green	0.81	1	Orange	0.81

CONCLUSION

In conclusion, we report this pharmacognostical, TPC and TFC data on the Rosa centifolia petals that can provide us a relevant source of information and contribute towards the standards for its identification, authentication and extensive medicinal use of the herbal plant part petals.

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None

Conflict of interest

The authors declare that there is no conflict of interest.

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