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(RESEARCH ARTICLE)

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Preliminary phytochemical analysis of leaves extracts of plant *Ougeinia oojeinensis*

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Abstract

Ougeinia oojeinensis, a member of the Fabaceae family commonly referred to as Tinsa, is primarily found in subtropical regions of India. It holds significant importance as a medicinal plant in deciduous forests and has been utilized in traditional medicine. This study aimed to assess the phytochemical composition of methanol and ethyl acetate extracts derived from the leaves of *Ougeinia oojeinensis*.

Various solvent extractions, including petroleum ether, methanol, and ethyl acetate, were employed to obtain leaf extracts of *Ougeinia oojeinensis*. Subsequently, a phytochemical screening of these extracts was conducted. The results of the screening revealed the presence of alkaloids, carbohydrates, terpenoids, flavonoids, tannins, phenolic compounds, saponins, and glycosides in the extracts.

Keywords: *Ougeinia oojeinensis*; Fabaceae; Phytochemical screening; Ethyl acetate; Leaf Extracts

1. Introduction

India has a rich cultural tradition of using plants for medicinal purposes, as documented in Ayurveda. Plants have long served as a valuable source of diverse chemical compounds, many of which exhibit significant pharmacological properties. Throughout history, plants have been a cornerstone of natural medicine, contributing to the preservation of human health. There is a growing preference for natural therapies to mitigate the potential adverse effects associated with some modern medications.

Medicinal plants are recognized for their immunomodulatory and antioxidant properties, which can lead to anticancer activities. In India, various cultural groups inhabiting diverse terrains maintain distinct cultures, religious practices, dietary habits, and a wealth of knowledge about traditional medicine. These groups have harnessed the power of medicinal herbs to combat an array of diseases (Samuelsson G., 1999). Natural products, particularly plants, have played a crucial role in the treatment of various illnesses for centuries, including cancers such as sarcoma, leukemia, lymphoma, and carcinoma.

Ougeinia oojeinensis, a member of the Fabaceae family, is known by several common names, including Tinsa, Sandan, and Panjan (Sharma, 2001; Singh, 2002). The leaves of *Ougeinia oojeinensis* have garnered particular attention due to their potential therapeutic properties. Phytochemical screening of *Ougeinia oojeinensis* involves the examination of methanolic leaf extracts to detect various phytochemicals. The results of phytochemical studies confirm the presence of alkaloids, carbohydrates, terpenoids, flavonoids, tannins, phenolic compounds, saponins, and glycosides in the methanolic extracts of *Ougeinia oojeinensis* leaves.

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These plant extracts have demonstrated a range of pharmacological activities, including anti-splasmodic, weak CNS depressant, anti-inflammatory, analgesic, antioxidant, anthelmintic, hepatoprotective, hypoglycemic, antidiabetic, and wound healing effects. Traditional uses of *Ougeinia oojeinensis* include the treatment of conditions such as jaundice, diarrhea, dysentery, urorrhagia, diabetes, verminosis, leprosy, leucoderma, hemorrhages, fevers, and ulcers (Gunasekaran, 2011; Samyal, 2014; Velmurugan, 2013; Verma, 2012). In this study, we present the findings of a screening for phytochemicals in ethyl acetate and methanol extracts of *Ougeinia oojeinensis* leaves.

The exploration of phytochemical diversity and the medicinal potential of *Ougeinia oojeinensis* leaf extracts is a significant endeavor. It not only sheds light on the plant's chemical composition but also underscores its traditional and contemporary relevance in herbal medicine. Understanding the bioactive compounds present in *Ougeinia oojeinensis* can pave the way for the development of novel therapeutic agents or the enhancement of existing natural remedies. This research contributes to the ongoing exploration of the plant kingdom's pharmaceutical potential and highlights the significance of preserving traditional medicinal knowledge.

1.1. 2. Plant Profile

- Botinical name : *Ougeinia oojeinensis*
- Kingdom: Plantae
- Order: Fabales
- Family: Fabaceae
- Subfamily: Faboideae
- Tribe: Desmodieae
- Species *Ougeinia oojeinensis* (Roxb)
- Common Name : Tinsa or sandan or Ujjain Desmodium tree

Figure 1 Plant of *Ougeinia oojeinensis*

2. Material and methods

2.1. Collection and identification of Medicinal Plants

To collect and identify *Ougeinia oojeinensis*_medicinal plants from Dharma tekri Chhindwawa, M.P. The species was identified by the local people during the time of collection and later on authentication was made by Mr. Mahesh Ghaywat Botanist, Danielson Degree college, *Chhindwara* , M.P. India.

2.2. Extraction of leaves of *Ougeinia oojeinensis*

The collected plant material was washed with water to remove other undesirable material and dried under shade. The air-dried leaves (120 gm) of *Ougeinia oojeinensis* were crushed. The crushed leaves extracted with petroleum ether, ethyl acetate and methanol by soxhlet extraction method .

2.2.1. Soxhlet Extraction

Powdered sample were placed in a thimble of Soxhlet apparatus. The extraction was carried out using different organic solvents; petroleum ether, ethyl acetate and methanol for 8-10 hours and 40-60^{o}C temperature of the heating mantle were adjusted (Alara *et al.,* 2019). After the extraction process, the extract of sample were filtered and concentrated to dryness. Extracts were collected in air tight container (Sahu *et al* 2009). Extraction yield of all extracts were calculated using the following equation below:

$$\text{Formula of Percentage yield } = \frac{\text{Actual yield}}{\boldsymbol{Theoretical\ yield}} \times \mathbf{100}$$

Where:

Weight of Extract Obtained: This is the actual weight of the extract collected after the Soxhlet extraction process.

Weight of Plant Material Used: This is the initial weight of the air-dried leaves of *Ougeinia oojeinensis* that you used for the extraction (in this case, 120 grams).

Both formulas, the one you provided earlier and this alternative one, will give you the same result for the percentage yield. They express the ratio of the actual yield of the extracted compounds to the amount of plant material used, scaled as a percentage. Choose the formula that best suits your presentation or reporting style.

2.3. Qualitative phytochemical analysis of plant extract

Plant extracts were obtained using standard methods as per Kokate (1986).The extracts were screened to identify the presence or absence of various active constituents like alkaloids, carbohydrates, terpenoids, flavonoids, tannins and phenolic compounds, saponins and glycosides.(Singh *et al.* 2015) 3.2.1. Tests for Carbohydrates

2.3.1. Molish Test

Two milliliters of the extract solution were mixed with 2 drops of alcoholic α-naphthol solution and 1 ml of concentrated sulfuric acid. The formation of a violet ring at the junction indicated the presence of carbohydrates.

2.3.2. Fehling's Test

One milliliter of Fehling's A and 1 ml of Fehling's B solutions were added to 1 ml of extract solution in a test tube. After heating in a water bath for 10 minutes, the presence of reducing sugar was indicated by the formation of a red precipitate.

2.3.3. Benedict's test

Equal volumes of Benedict's reagent and extract solution were mixed and heated in a water bath for 5-10 minutes. The presence of reducing sugar was determined by color changes (green, yellow, or red).

2.3.4. Tests for Alkaloids

Dilute hydrochloric acid was added to the plant extract solution, followed by vigorous shaking to ensure proper mixing. The mixture was then filtered to obtain the alkaloid-containing filtrate.

2.3.5. Mayer's Test

A few drops of Mayer's reagent were introduced into a test tube and 2-3 ml of the above-mentioned filtrate were added carefully along the sides of the test tube.The presence of alkaloids was indicated by the formation of a white or creamy precipitate.

2.3.6. Hager's Test

In a separate test tube, a few drops of Hager's reagent were added and 1-2 ml of the above-mentioned filtrate were introduced.The presence of alkaloids was confirmed by the formation of a yellow-colored precipitate.

2.3.7. Wagner's Test

One milliliter of the plant extract was taken, and 2 ml of Wagner's reagent (Iodine in Potassium Iodide) was added to it.The presence of alkaloids was revealed by the formation of a reddish-brown precipitate**.**

2.4. Tests for Triterpenoids and Steroids

2.4.1. Salkowski's Test

In a test tube, the plant extract solution was taken and Chloroform was added to the extract solution, and the mixture was shaken thoroughly.The solution was then filtered to separate the layers.To the filtrate, a few drops of concentrated sulfuric acid were added carefully, and the tube was shaken gently.The mixture was allowed to stand undisturbed.The presence of sterols was indicated if the lower layer turned red.

The presence of triterpenes was indicated by the presence of a golden-yellow layer at the bottom.

2.4.2. Libermann- Burchard's Test

The plant extract solution was taken and few drops of chloroform were added to the plant extract solution to dissolve it. To the dissolved extract solution, 3 ml of acetic anhydride and 3 ml of glacial acetic acid were added in a test tube. The test tube was gently warmed and then allowed to cool under a tap. Next, drops of concentrated sulfuric acid were carefully added along the sides of the test tube.

The presence of steroids was indicated if a brown ring formation occurred at the junction of the two layers .If the upper layer turned green, it was suggestive of the presence of steroids.The presence of triterpenoids was indicated if there was the formation of a deep red color.

2.5. Tests for Flavonoids

2.5.1. Lead Acetate Test

The plant extract solution was prepared and a few drops of lead acetate solution were added to the plant extract solution. The presence of flavonoids was indicated if there was the development of a yellow precipitate in the solution.

2.5.2. Alkaline Reagent Test

In a separate test tube, extract solution was treated with few drops of sodium hydroxide. The presence of flavonoids indicates by the formation of intense yellow color, which becomes colorless on addition of few drops of dilute acid.

2.6. Tests for Tannin and Phenolic compounds

2.6.1. Ferric Chloride Test

Dissolve a small amount of extract in distilled water. Add 2 ml of a 5% ferric chloride (FeCl3) solution to this solution.The presence of phenolic compounds, especially tannins, is indicated by the formation of a blue, green, or violet color in the solution.

2.6.2. Lead Acetate Test

In distilled water little amount of extract was dissolved. To this solution few drops of lead acetate solution was add on. The Formation of white precipitate indicates the presence of phenolic compounds.

2.6.3. Gelatin Test

Into the distilled water some quantity of extract was dissolved. To this solution 2 ml of 1% gelatin solution containing 10% sodium chloride was added. Development of white precipitate depicts the presence of phenolic compounds.

2.7. Tests for Saponins

2.7.1. Froth Test

With the help of distilled water the extract was diluted and shaken in graduated cylinder for 15 minutes. The formation of layer of foam shows the presence of saponins.

2.8. Tests for Fats and Oils

2.8.1. Solubility test

Add few ml of chloroform to 1-2 ml of the alcoholic solution of extract, and solubility was observed. Add few ml of 90% ethanol to 2-3 ml of the alcoholic solution of extract, and solubility was observed.

2.9. Tests for Protein and Amino acids

2.9.1. Biuret's Test

In a test tube, extract solution was added to 1 ml of 10% sodium hydroxide solution and heated. A drop of 0.7% copper sulphate solution was mixed to the above mixture. The production of violet or pink colour specifies the presence of proteins.

2.9.2. Ninhydrin Test

3 drops of 5% Ninhydrin solution was added to 3 ml of the extract solution and heated in a water bath for 10 minutes. The formation of blue colour shows the presence of amino acids

2.10. Tests for Glycosides

2.10.1. Borntrager's Test

Dilute sulphuric acid was added to 3 ml of *extract solution*, boiled for 5 minutes and filtered. Equal volume of benzene or chloroform was added to the cold filtrate and mixed it well. The organic solvent layer was isolated and ammonia was added to it. Presence of anthraquinone glycosides is confirmed by the formation of pink to red color in ammonical layer.

2.10.2. Legal's Test

In pyridine 1 ml of *extract* solution was dissolved. 1 mL sodium nitropruside solution was added, and the solution was made alkaline using a 10% sodium hydroxide solution. Formation of pink to blood red color shows the presence of Cardiac glycosides.

2.10.3. Keller-Killiani Test

In a test tube added 2 ml of extract solution, 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride. By the side of the test tube mix carefully 0.5 ml of concentrated sulphuric acid. The presence of Cardiac glycosides is depicted by formation of blue color in the acetic acid layer.

3. Results and discussion

The air-dried leaves (120 gm) of *Ougeinia oojeinensis* are subjected to Soxhlet Extraction with e were carried out using different organic solvents; petroleum ether, ethyl acetate and methanol and the yield was 30.45 gm. Further successive fractionation (separation technique) of methanol extract was done by using different solvents of increasing polarity & yields were Ethyl acetate (6.23 gm) and methanol (24.22 gm). The ethyl acetate and methanol extracts of *Ougeinia oojeinensis* leaves are rich in a variety of phytoconstituents, including alkaloids, terpenoids, flavonoids, tannins, phenolic compounds, saponins, glycosides, and carbohydrates. Ethyl acetate fractions showed the presence of alkaloids, terpenoids, flavonoids, tannins and phenolic compounds. Methanol fractions showed the presence of carbohydrate, alkaloids, terpenoids, flavonoids, tannins and phenolic compounds, saponins and glycosides. These compounds have diverse biological activities and can be of interest for further pharmacological or medicinal research .It's important to note that the presence of these phytoconstituents can contribute to the plant's medicinal properties, and further studies may be conducted to isolate and characterize specific compounds for potential therapeutic applications.

3.1. Plant Collection

Table 1 Plant collection

S. No.	Plant name	Plant part used	Weight
1.	*Ougeiniaoojeinensis*	Leaf	120gm

3.2. Percentage yield

Table 2 Percentage yield of *Ougeinia oojeinensis*

S. No.	Solvent	Color of extract	Theoretical weight (gm)	Yield in gms	% Yield
1.	Pet. Ether	Transparent	120	No yield	-
2.	Ethyl acetate	Green	108.32	6.23	5.75
3.	Methanol	Green	96.41	24.22	25.12

3.3. Solubility determination

Table 3 Solubility Determination of *Ougeinia oojeinensis* Extract

S. No.	Solvent	Ethyl acetate	Methanol
1.	Water	Insoluble	Soluble
2.	Ethanol	Insoluble	Soluble
3.	Chloroform	Soluble	Slightly soluble
4.	DMSO	Soluble	Soluble
5.	Petroleum Ether	Slightly soluble	Insoluble

3.4. Qualitative Phytochemical Analysis of *Ougeinia oojeinensis* extracts

Table 4 Phytochemical analysis of *Ougeinia oojeinensis* extracts

S. No.	Experiment	Result	
		Ethyl acetate	Methanol
Test for Carbohydrates			
1.	Molisch's Test	-	+
2.	Fehling's Test	-	+
3.	Benedict's Test	-	+
4.	Bareford's Test	-	+
Test for Alkaloids			
1.	Mayer's Test	+	+
2.	Hager's Test	+	+
3.	Wagner's Test	+	+
4.	Dragendroff's Test	+	+
Test for Terpenoids			
1.	Salkowski Test	+	+
2.	Libermann-Burchard's Test	+	-

Test for Flavonoids			
1.	Lead Acetate Test	+	+
2.	Alkaline Reagent Test	+	+
3.	Shinoda Test	+	+
Test for Tannins and Phenolic Compounds			
1.	$FeCl_3$ Test	+	+
2.	Lead Acetate Test	+	+
3.	Gelatine Test	+	+
4.	Dilute Iodine Solution Test	+	+
Test for Saponins			
1.	Froth Test	-	+
Test for Protein and Amino acids			
1.	Ninhydrin Test	-	-
2.	Biuret's Test	-	-
3.	Million's Test	-	-
Test for Glycosides			
1.	Legal's Test	-	+
2.	Keller Killani Test	-	+
3.	Borntrager's Test	-	+

+ = present, - = absent.

4. Conclusion

The study on the leaves of *Ougenia oojeinensis* (Roxb.) Hochr. has revealed a diverse range of phytoconstituents, including alkaloids, carbohydrates, terpenoids, flavonoids, tannins, phenolic compounds, saponins, and glycosides in both methanol and ethyl acetate extracts. These findings suggest that *O. oojeinensis* possesses a rich reservoir of potentially bioactive compounds. While qualitative tests have confirmed the presence of these compounds, further research is required to isolate, characterize, and quantify specific bioactive components from the extracts. Additionally, the establishment of quality standards for these extracts will be essential for future investigations and potential medicinal applications.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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