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Research

In-Vitro Anti-inflammatory and Antioxidant Activity of Ougeinia oojeinensis Bark Extract

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Abstract

Background: *Ougeinia oojeinensis* is a plant known for its medicinal properties. In this study, we aimed to investigate the phytochemical composition and pharmacological activities of the bark extracts of *Ougeinia oojeinensis.*

Methods: The bark of *Ougeinia oojeinensis* was extracted using the soxhlation method with three different solvents: methanol, ethyl acetate, and petroleum ether. The percentage yield of each extract was determined. Phytochemical screening was performed to identify the presence of various bioactive compounds, including Carbohydrates, Flavonoids, Tannins, Phenolics, and Fats. The extracts were further evaluated for their Anti-Inflammatory and Antioxidant activities using appropriate in vitro assays.

Results: Among the three solvents, the methanol extract exhibited the highest percentage yield, indicating its efficient extraction of bioactive compounds from the bark. Phytochemical screening revealed the presence of carbohydrates, flavonoids, tannins, phenolics, and fats in the methanolic and ethyl acetate extracts. The petroleum ether extract contained fats as the sole detected compound. In terms of pharmacological activities, the methanolic extract showed the most significant Anti-Inflammatory and Antioxidant activities, suggesting its potential therapeutic application.

Keywords: : *Ougeinia oojeinensis,* Anti-inflammatory, Antioxidant, phytochemicals and egg albumin denaturation assay.

INTRODUCTION

Ougeinia oojeinensis is a deciduous tree belonging to the*Fabaceae* family. Its various parts, including leaves, bark, and flowers, have been extensively used in traditional Indian medicine for centuries to alleviate a wide array of health conditions. Among its traditional uses, *Ougeinia oojeinensis*has been employed as an analgesic, anti-inflammatory, antipyretic, and antimicrobial agent.

While traditional knowledge has attributed these therapeutic properties to the plant, scientific validation through rigorous investigation is essential to substantiate its potential medicinal applications (1).

Oxidative stress is a condition characterized by an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defence mechanisms. Excessive ROS can lead to cellular damage and contribute to the pathogenesis of several chronic diseases, including cardiovascular disorders, neurodegenerative conditions, and cancer. On the other hand, inflammation is a natural defence mechanism triggered by the immune system in response to injury or infection. However, chronic inflammation can lead to tissue damage and the development of inflammatory disorders, such as arthritis, asthma, and inflammatory bowel disease(2).

In-vitro assays serve as valuable tools in the initial screening of plant extracts to assess their bioactivity. These assays enable researchers to understand the mechanisms of action and evaluate the potential therapeutic benefits of natural products. Therefore, the present study aimed to investigate the anti-inflammatory and antioxidant activities of *Ougeinia oojeinensis*extracts through a series of in vitro assays(3).

MATERIALS AND METHODS

Thebark of the plantwascollected, and authenticated by

Dr.Shivakumar Singh P, Department of Botany, Palamuru University, Mahabubnagar, Telangana, India.The powdered bark was subjected to shade drying to remove excess moisture and grind them into a fine powder.25 grams of powdered *Ougeinia oojeinensis*plant material was weighed accurately and placed inside a thimble made of filter paper. The loaded thimble was placed into the Soxhlet extractor using methanol, ethyl acetate and petroleum ether as solvents. The extraction process was continued for 72 hours until the extraction reaches a satisfactory level (4).

Fig 1: Soxhalation of *Ougeinia oojeinensis*

Recovery of extract

After the extraction process, the extracted solution was collected and concentrated. The apparatus was disconnected and transfer the extract from the round-bottom flask to a separate container. The extract was concentrated by evaporation at room temperature.

Fig 2:Concentration of extracts at room temperature

Evaluation of extract

After the extraction process, the obtained extract from *Ougeinia oojeinensis* needs to be evaluated to determine its phytochemical composition and potential biological activities. The percentage yield is a useful parameter in the field of natural product extraction, as it provides a measure of the efficiency of the extraction process. The percentage yield is the ratio of the weight of the obtained extract to the weight of the plant material, expressed as a percentage. It indicates the proportion of desired compounds successfully extracted from the plant material. Weigh the plant material using a balance and record the weight in grams (g). After completing the extraction, collect the extract obtained.Weigh the extract using a balance and record the weight in grams (g).

Percentage Yield = (Weight of Extract / Weight of Plant Material) × 100

Antioxidant Activity

Antioxidant assays are valuable tools for assessing the antioxidant capacity of natural compounds and extracts. The phosphomolybdenum assay was used to measure the total antioxidant capacity of a sample (5).

The antioxidant assay was performed by using the phosphomolybdenum reagent, In a clean test tube, 1 ml of the sample solution with 9 ml of the prepared phosphomolybdenum reagent was taken. It was thoroughly mixed to ensure a proper reaction.The reaction mixture was incubated at room temperature for 90 minutes. This incubation allows the antioxidants in the sample to interact with the phosphomolybdate reagent and reduce it.After the incubation period, a spectrophotometer was used to measure the absorbance of the reaction mixture at wavelength 695 nm. theblank cuvette was made by taking the same volumes of reagent but without the sample, and absorbance was measured and taken as a reference.To calculate the antioxidant capacity, subtract the absorbance of the blank from the absorbance of the sample to eliminate any background interference. Compare the absorbance value of the sample to a standard using known concentrations(1mg/ml) of Ascorbic acid using the formula given below (6).

$$\% \text{ Antioxidant activity} = \frac{Absorbance(control) - Absorbance(sample)}{Absorbance(control)} \text{ x } 100$$

Anti-inflammatory Activity

Anti-inflammatory activity was performed by using egg albumin was used as a protein. Denaturation of protein is induced by keeping the reaction mixture at 70°C in a water bath for 10 minutes.The reaction mixture consists of various concentrations of plant extract 1000 µL (100-500 µg/ml), 200 µL of egg albumin or 450 µL (5% w/v aqueous solution) egg albumin, 1400 µL of phosphate buffered saline. Distilled water instead of extracts with the above mixture is used as a negative control. Afterwards, the mixtures are incubated at 37 °C for 15 min and then heated at 70°C for 5 min.After cooling under running tap water, their absorbances are measured at 660 nm. Ibuprofen is taken as a positive control. The experiment is carried out in triplicates and percent inhibition for protein denaturation is calculated using the following equations (7).

% Inhibition of denaturation = (1-D/C) X100.

Where D is the absorbance of the test sample and C is the absorbance of the negative control (without the test sample or reference drug)

RESULTS AND DISCUSSION

Percentage Yield of Extracts

The extraction of *Ougeinia oojeinensis* bark using the Soxhlet extraction method resulted in different percentage yields for different solvents. The methanolic extract exhibited the highest percentage yield at 41.2%, followed by the ethyl acetate extract at 3.16% and the petroleum ether extract at 1.64%.The higher percentage yield of the methanolic extract suggests that methanol is a more efficient solvent for extracting the bioactive constituents from *Ougeinia oojeinensis* bark compared to ethyl acetate and petroleum ether. This could be attributed to the solubility of the phytochemicals present in the bark, as well as the polarity of the solvent. Methanol is a polar solvent, which can efficiently extract a wide range of polar and semi-polar compounds.

The lower percentage yields of the ethyl acetate and petroleum ether extracts indicate that these solvents may be less effective in extracting the bioactive constituents from the bark.

$$\text{Percentage Yield} = \frac{Weight\ of\ Extract}{Weight\ of\ plant\ material} \times 100$$

Table 1: Percentage yield of Methanolic, Ethyl acetate and petroleum ether extracts of *Ougeinia oojeinensis*

Extracts	Weight of plant material (gm)	Weight of extract (gm)	Percentage yield (%)
Methanolic extract	25	10.30	41.2
Ethyl Acetate Extract	25	0.79	3.16

Petroleum ether Extract	25	0.41	1.64

Phytochemical Analysis

Table 2: Phytochemical screening of Methanolic, Ethyl acetate and petroleum ether extracts of *Ougeinia oojeinensis*

Phyto-constituent	Tests performed	Extracts		
		Methanolic	Ethyl-acetate	Petroleum ether
Carbohydrates	Molisch's test	+	+	-
Proteins	Millon's test	-	-	-
Amino Acids	Ninhydrin test	-	-	-
Steroids	Salkowski reaction	-	+	-
Glycosides	General test	-	-	-
Flavanoids	Shinoda test	+	+	-
Tannins and phenolic compounds	a) 5% $FeCl_3$ solution test	+	+	-
	b) Lead acetate solution test	+	+	-
Alkaloids	a)Dragendorff's test	-	-	-
	b) Mayer's test	-	-	-
Fats and oils	Translucent test	+	+	+

Anti-inflammatory activity

The egg albumin denaturation assay is a commonly used method to evaluate the anti-inflammatory activity of test compounds or extracts. In this study, the assay was performed to assess the anti-inflammatory potential of the *Ougeinia oojeinensis* bark extracts, along with the standard drug ibuprofen.The results showed that ibuprofen, a well-known anti-inflammatory drug, exhibited a dose-dependent inhibition of egg albumin denaturation. At concentrations of 10µg/ml, 30µg/ml, and 100µg/ml, the percentage inhibition was found to be 50.96%, 55.40%, and 68.91% respectively. These results validate the effectiveness of the assay and serve as a positive control for the study.The methanolic extract of *Ougeinia oojeinensis* bark demonstrated significant anti-inflammatory activity as well. At concentrations of 10µg/ml, 30µg/ml, and 100µg/ml, the percentage inhibition was found to be 40.4%, 47.2%, and 51.4% respectively. These findings suggest that the methanolic extract possesses anti-inflammatory properties, albeit to a lesser extent compared to ibuprofen.Similarly, the ethyl acetate extract exhibited considerable anti-inflammatory activity. The percentage inhibition at concentrations of 10µg/ml, 30µg/ml, and 100µg/ml was 35.4%, 44.1%, and 46.8% respectively. These results indicate that the ethyl acetate extract has the potential to inhibit protein denaturation and, thus, exhibit anti-inflammatory effects.The petroleum ether extract also displayed anti-inflammatory activity, albeit with a relatively lower percentage of inhibitions. At concentrations of 10µg/ml, 30µg/ml, and 100µg/ml, the percentage inhibition was found to be 32.7%, 38.5%, and 40.3% respectively. Although the petroleum ether extract demonstrated a relatively weaker anti-inflammatory effect compared to the methanolic and ethyl acetate extracts, it still exhibited some level of inhibition.

$$\textbf{(\%)Percentage inhibition} = 1 - \frac{D}{C} X 100$$

Where **D** is the absorbance of the test sample and **C** is the absorbance of negative control **C=2.524**

Table 3:Percentage protein inhibition of Methanolic, Ethyl acetate and petroleum ether extracts of *Ougeinia oojeinensis*

Sample	Concentration (µg/ml)	Absorbance	Percentage inhibition (%)
Ibuprofen	10	1.238	50.96
	30	1.128	55.40
	100	0.785	68.91
Methanolic Extract	10	1.505	40.4
	30	1.333	47.2
	100	1.227	51.4
Ethyl Acetate Extract	10	1.633	35.4
	30	1.412	44.1
	100	1.343	46.8
Petroleum Ether Extract	10	1.699	32.7
	30	1.553	38.5
	100	1.509	40.3

Antioxidant Activity

The phosphomolybdenum reagent assay is a commonly used method to evaluate the antioxidant activity of plant extracts or compounds. In this study, the assay was performed to assess the antioxidant potential of the *Ougeinia oojeinensis* bark extracts,

using ascorbic acid as the standard antioxidant.The results revealed that the methanolic extract of *Ougeinia oojeinensis* bark exhibited a significant antioxidant activity with a percentage antioxidant activity of 39.7%. This indicates that the methanolic extract possesses strong antioxidant properties, as it demonstrated a high ability to scavenge free radicals and reduce oxidative stress. The high antioxidant activity of the methanolic extract suggests the presence of bioactive compounds with potent antioxidant effects.The ethyl acetate extract also showed notable antioxidant activity, albeit to a lesser extent compared to the methanolic extract. The percentage antioxidant activity of the ethyl acetate extract was found to be 28.3%. This suggests that the ethyl acetate extract contains compounds that can effectively neutralize free radicals and provide protection against oxidative damage.On the other hand, the petroleum ether extract exhibited relatively lower antioxidant activity with a percentage of 13.7%. Although the petroleum ether extract demonstrated a weaker antioxidant effect compared to the methanolic and ethyl acetate extracts, it still displayed some level of antioxidant activity.

$$\textbf{(\%)Percentage Antioxidant activity } \frac{\boldsymbol{Absorbance(control)-Absorbance(sample)}}{\boldsymbol{Absorbance(control)}} \textbf{ x 100}$$

Table 4:Percentage antioxidant activity of Methanolic, Ethyl acetate and petroleum ether extracts of *Ougeinia oojeinensis*

Sample	Absorbance	Percentage Antioxidant activity (%)
Ascorbic acid	2.948	---
Methanolic extract	1.777	39.7
Ethyl acetate extract	2.113	28.3
Petroleum ether extract	2.542	13.7

CONCLUSION

In conclusion, this *In-Vitro* Anti-inflammatory and Antioxidant activity of *Ougeinia oojeinensis* bark extract provides valuable insights into its potential medicinal properties. The findings of this study highlight the promising medicinal properties of *Ougeinia oojeinensis*bark. The bark extracts exhibit a diverse range of bioactive compounds, significant anti-inflammatory activity, and notable antioxidant potential. These results support the traditional use of this plant in herbal medicine.

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