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PRELIMINARY PHYTOCHEMICAL AND ANTI-INFLAMMATORY SCREENING OF OROXYLUM INDICUM LEAVES

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Abstract

Background

Herbal plants, rich in diverse phytoconstituents, offer a promising alternative pathway for developing safer, more effective anti-inflammatory drugs with fewer adverse effects.

Objectives

To assess the preliminary phytochemical and anti-inflammatory activities of the leaves of Oroxylum indicum.

Method

Ash value, extractive value, and chromatographic analysis were performed in accordance with official methods. Preliminary phytochemical screening was carried out to find out the presence of phytoconstituents category. The carrageenan-induced model was used to evaluate the anti-inflammatory activity.

Results

The total ash, acid-insoluble, water-soluble, and sulphated ash values were found to be 6.75%, 0.177%, 2.19%, and 6.09%, respectively. In comparison to alcohol, the water-soluble extractive value was higher. Preliminary phytochemical screening of different extracts showed the presence of alkaloids, carbohydrates, glycosides, phenolic compounds, tannins, sterols, saponins, proteins, and flavonoids. Chloroform extract showed the highest anti-inflammatory activity (>70% inhibition), comparable to the standard drug diclofenac sodium, followed by acetone (58.23%), ethanol (50.00%), petroleum ether (46.84%), and aqueous extracts (3.80%).

Conclusion

The chloroform extract of the leaves of Oroxylum indicum showed maximum anti-inflammatory activity.

Keywords: Oroxylum indicum, anti-inflammatory, diclofenac sodium.

Introduction

Plants have served as source of food and medicine for millennia1. Traditional plants played significant role in the development of new drugs2. Oroxylum indium, a member of the Bignoniaceae family, is a deciduous tree up to a height of 12 m, commonly found up to an altitude of 1200 m in moist places of the forest. Traditionally, the root bark of this plant is used in diarrhoea, dysentery, rheumatoid arthritis, and fever. Leaf decoction is used to treat stomachache and rheumatism. Mature fruits are reported to be used in jaundice, cough, bronchitis, flatulence, and leukoderma. The seed of this species is used as a purgative3. Phytochemically flavonoids4, naphthalene compounds5, tannic acid, scutellarein-7-rutinoside3, baicalein6, baicalein-7-o-glucoside, chrysin7. Pharmacologically immunostimulant8, antimicrobial, anti-inflammatory9, antiulcer10, antimutagenic11, antioxidant12 activities reported in various studies.

The transition from conventional NSAIDs to herbal anti-inflammatory agents represents an important shift in therapeutics. NSAIDs have proven efficacy; their cardiovascular, gastrointestinal, and renal side effects, combined with high costs and symptom recurrence, create an urgent clinical need. Herbal plants, rich in diverse phytochemical compounds that work synergistically, offer a promising alternative pathway for developing safer, more effective anti-inflammatory drugs with fewer adverse effects.13 Based on the importance of herbal drugs, the present study aimed to assess the preliminary phytochemical and anti-inflammatory activities of the leaves of Oroxylum indicum.

Material And Methods

Collection of Plant Material

The leaves of Oroxylum indicum were collected from Susheela Tiwari Herbal Garden, Rishikesh, Uttarakhand and identified by Prof. G.S. Bisht, Department of Biotechnology, SBS PGI, Balawala, Dehradun. The fresh samples were dried at room temperature for 15 days. After drying, the leaves were crushed into powder and weighed.

Physiochemical analysis

Physiological parameters, such as ash and extractive values, were determined in accordance with WHO guidelines.14

Preparation of Extract & Preliminary Phytochemical Screening

Petroleum ether, chloroform, acetone, ethanol, and water were used to extract 150 g of coarse powder of leaves of Oroxylum indicum. Extraction was carried out by the Soxhlet apparatus. Before each extraction with the next solvent, the powdered material was dried in a hot-air oven at below 50°C. The individual fractions obtained were concentrated under reduced pressure in a water bath and weighed. Obtained extracts were evaluated by preliminary phytochemical screening to determine the presence of chemical constituents.15

Chromatographic Analysis

TLC plates coated with a slurry of silica gel G having thickness of 0.25mm were used for the chromatographic analysis. H2SO4 (05%) was used as visualizing agent to detect the presence of phytoconstituents. The Rf value was calculated.15

In vivo Anti-inflammatory activity

Albino Wistar rats (180-240 g) of either sex were used for the study. The animals were kept in large, clean polypropylene animal cages in room temperature for one week before experiments and were feed a standard diet. Animals fasted overnight with free access to water prior to the experiment. A total of 35 rats were divided into 7 groups of 5 rats each. Group I served as the normal control and received only the normal saline (10mg/ml). Group II received diclofenac sodium(0.5mg/ml). Group III, IV, V, VI, and VII received petroleum ether, chloroform, acetone, ethanol, and water extract of leaves of Oroxylum indicum. The dose was selected 100 mg/kg for all the extracts. Test drugs were given orally after 0.1(w/v) carrageenin, which was injected subcutaneously into the plantar region of the right hind paw of the rats of each group. Initial and 2 hr after carrageenin injection, paw volume was measured by the mercury displacement method. The difference between the initial and final volume indicated the volume of inflammation. The percentage of inhibition was calculated from the volume value obtained.16

Results And Discussion

Determination of the ash value of a sample gives an idea about the earthy matter, inorganic composition, and other impurities present along with the drug. The total ash value was found to be 6.75%, indicating the total amount of inorganic residue remaining after incineration of the leaf sample. The acid-insoluble ash was 0.177%, representing a relatively small proportion of siliceous and other acid-insoluble inorganic matter. The water-soluble ash was 2.19%, indicating the proportion of the total ash that is soluble in water. The sulphated ash value was 6.09%. Results are tabulated in Table 1.

TABLE 1: Results of Ash Value Determination

Total ash	Acid-insoluble ash	Water-soluble ash	Sulphated ash
6.75	0.177	2.19	6.09

Extractive values are primarily useful for determining extractable constituents in a crude drug. The alcohol-soluble and water-soluble extractive values were 12.67% and 31.71%, respectively. The higher water-soluble extractive value suggests the presence of appreciable amounts of polar phytoconstituents, such as sugars, glycosides, tannins, certain phenolic compounds, proteins, and other water-soluble substances. In contrast, the comparatively lower alcohol-soluble extractive value indicates a relatively smaller proportion of constituents readily extractable in alcohol under the experimental conditions. Higher water-soluble extractive value also support the traditional use of the plant material in aqueous preparations such as infusions or decoctions. However, the extractive value alone does not establish pharmacological activity, and further phytochemical profiling is required to identify the compounds responsible for the observed extractability.

TABLE 2: Results of Extractive Value Determination

Alcohol soluble extractive value	Water-soluble extractive value
12.67 %	31.71%

The powdered leaf of Oroxylum indicum was successively extracted using solvents of different polarities, namely petroleum ether, chloroform, acetone, ethanol, and water. The duration of extraction and corresponding practical yields are presented in Table 4. The highest practical yield was found in water, indicating the presence of polar and water-soluble constituents, such as sugars, glycosides, tannins, certain phenolic compounds, proteins, and amino acids. The second-highest practical yield was with the solvent ethanol, although the ethanol yield was considerably lower than that of water, the ethanolic extract may contain a chemically diverse range of secondary metabolites such as flavonoids, glycosides, alkaloids. The chloroform extract yielded 6.40 g, while petroleum ether yielded 5.62 g. These relatively lower yields may reflect the comparatively smaller proportion of non-polar and moderately non-polar constituents in the plant material. Petroleum ether predominantly extracts lipophilic substances such as fats, oils, waxes, pigments, and other non-polar compounds, whereas chloroform can extract moderately lipophilic constituents. The lowest yield was observed with acetone (2.44 g). This comparatively low recovery may indicate that fewer constituents with suitable solubility in acetone were present in the plant material under the extraction conditions used.

Table 4: Practical yield of the extract in Different Solvents

Solvent	No. of Hr. of extraction	Practical yield in g
Petroleum ether	18Hr.	5.62
Chloroform	15 Hr.	6.40
Acetone	12 Hr.	2.44
Ethanol	12 Hr.	12.85
Water	20 Hr.	45.13

The results of the qualitative phytochemical analysis are presented in Table X. Alkaloids were not detected in the petroleum ether, chloroform, or acetone extracts in the tests performed. However, the ethanol and aqueous extracts showed positive reactions with Dragendorff's and Hager's tests (++), while Wagner's test showed a weaker positive reaction (+). The occurrence of positive reactions predominantly in the polar extracts suggests that the alkaloidal constituents, if present, are preferentially extracted in polar solvents. The tests for glycosides and carbohydrates demonstrated a variable distribution among the extracts. Fehling's test was positive in the chloroform and acetone extracts (+) and showed a stronger reaction in the ethanol extract (+++), while the aqueous extract showed a moderate reaction (++). Molisch's and Barfoed's tests were positive only in the aqueous extract, whereas Benedict's and Legal's tests were positive in the ethanol and aqueous extracts. These findings indicate the presence of carbohydrate-related and/or glycosidic constituents, particularly in the more polar extracts. The strong response to Fehling's test in the ethanolic extract suggests a relatively greater abundance of reducing constituents in this extract. For phenolic compounds and tannins, the Vanillin-HCl test showed a positive reaction only in the ethanol extract (+). Ferric chloride testing, however, was negative in all extracts. Thus, the present results provide limited evidence for phenolic/tannin constituents, with the positive Vanillin-HCl reaction suggesting that certain phenolic or tannin-related constituents may be present in the ethanolic extract. Sterols exhibited a distinct distribution among the

extracts. Salkowski's test was positive in the chloroform extract (+), whereas Liebermann's test showed positive reactions in the petroleum ether (++) and chloroform (+++) extracts. The stronger reaction observed in the chloroform extract suggests a relatively greater presence of sterol-type constituents in this fraction. Saponins were detected by the foam test in the chloroform, ethanol, and aqueous extracts, with responses of +, ++, and +++, respectively. The strongest reaction was observed in the aqueous extract, indicating a comparatively higher abundance of foam-forming saponin constituents in the water extract. The tests for proteins and amino acids were largely negative. Millon's and Ninhydrin tests showed negative reactions in all extracts. However, Biuret's test was positive in the acetone, ethanol, and aqueous extracts (+). This suggests the possible presence of proteinaceous constituents or peptide-related substances in these extracts, although the absence of response in the other protein/amino-acid tests indicates that these findings should be interpreted cautiously and confirmed using more specific analytical methods. Flavonoids were detected mainly in the chloroform, acetone, and ethanol extracts. Ferric chloride, zinc-HCl reduction, and alkaline reagent tests generally produced ++ reactions in these extracts. The petroleum ether and aqueous extracts were negative in the reported flavonoid tests. The consistent positive reactions in the chloroform, acetone, and ethanol fractions suggest the presence of flavonoid-type constituents with suitable solubility in these solvents. Preliminary phytochemical screening showed that the ethanol extract exhibited the broadest range of positive reactions, particularly for alkaloids, carbohydrates/glycosides, phenolic/tannin-related constituents, proteins, and flavonoids. The aqueous extract was particularly rich in saponins and carbohydrate/glycoside-related constituents, while the chloroform and petroleum ether extracts showed greater evidence of sterol-type constituents.

TABLE 5: Results of Preliminary Phytochemical Screening

Category of Phytoconstituents	Identification Test	Petroleum ether extract	Chloroform extract	Acetone extract	Ethanol extract	Water extract
Alkaloids	Mayer’s test	-	-	-	-	-
	Dragendorff’s test	-	-	-	++	++
	Hager’s test	-	-	-	++	++
	Wagner’s test	-	-	-	+	+
Glycosides and Carbohydrate	Fehling test	-	+	+	+++	++
	Molisch’s Test	-	-	-	-	+
	Barfoed test	-	-	-	-	+
	Benedict test	-	-	-	+	+
	Legal test	-	-	-	+	+
Phenolic compounds and tannins	Vanillin HCl	-	-	-	+	-
	Ferric Chloride test	-	-	-	-	-
Sterols	Salkowski test	-	+	-	-	-

	Liebermann test	++	+++	-	-	-
Saponin	Foam test	-	+	-	++	+++
Protein and amino acid	Milon's test	-	-	-	-	-
	Ninhydrin test	-	-	-	-	-
	Biuret test	-	-	+	+	+
Flavonoids	Ferric chloride test	-	++	++	++	-
	Zinc hydrochloric acid reduction test	-	+	++	++	-
	Alkaline reagent test	-	++	++	++	-

Chromatographic analysis of the different solvent extracts revealed multiple spots with distinct Rf values, indicating the presence of several chemically diverse constituents. The petroleum ether extract showed four spots with Rf values ranging from 0.138 to 0.841, while the chloroform extract showed four spots ranging from 0.176 to 0.704. The acetone extract exhibited three spots with Rf values of 0.250, 0.450, and 0.742. The ethanol extract showed four spots with Rf values ranging from 0.114 to 0.714. The variation in Rf values among the extracts indicates differences in the polarity and composition of their phytoconstituents.

TABLE 6: Results of Thin Layer Chromatography

Extract	**Mobile Phase**	**Distance travelled by spot (cm)**	**Distance travelled by solvent (cm)**	**Rf Value**
Petroleum ether	Petroleum ether: Ethyl acetate (80:20)	Spot A: 2.0	14.5	0.138
		Spot B: 4.5	14.5	0.310
		Spot C: 6.4	14.5	0.441
		Spot D: 12.2	14.5	0.841
Chloroform	Chloroform 100 %	Spot A: 2.5	14.2	0.176
		Spot B: 4.8	14.2	0.338
		Spot C: 8.0	14.2	0.563
		Spot D: 10.0	14.2	0.704
Acetone	Chloroform: Methanol (95:5)	Spot A: 3.0	12.0	0.250
		Spot B: 5.4	12.0	0.450
		Spot C: 8.9	12.0	0.742
Ethanol	Chloroform: Methanol (90:10)	Spot A: 1.2	10.5	0.114
		Spot B: 2.2	10.5	0.210
		Spot C: 4.5	10.5	0.429
		Spot D: 7.5	10.5	0.714

The anti-inflammatory activity of different extracts of leaves of Oroxylum indicum were evaluated using the carrageenan-induced paw edema model, with paw swelling measured as an indicator of acute inflammation. The percentage inhibition of paw edema was calculated in comparison with the control group. The control group exhibited a mean paw swelling of 0.316, which was considered the reference value for calculating the percentage inhibition. Among the tested samples, mean paw swelling ranged from 0.078 to 0.304, corresponding to 3.80–75.32% inhibition of paw edema.

The standard diclofenac sodium showed mean paw swelling of 0.078 exhibited the highest anti-inflammatory activity, with 75.32% inhibition, followed by the chloroform extract with a mean paw swelling of 0.088, which showed 72.15% inhibition. The extracts with mean paw swelling values of acetone extract (0.132), ethanol extract (0.158), and petroleum ether extract (0.168) produced 58.23%, 50.00%, and 46.84% inhibition, respectively. In contrast, the sample with a mean paw swelling of water extract (0.304) showed only 3.80% inhibition, indicating comparatively weak activity.

The inflammatory response produced by carrageenan involves the sequential release of several inflammatory mediators, such as histamine and serotonin, and other inflammatory mediators. Therefore, inhibition of carrageenan-induced paw edema suggests that a test sample may interfere with one or more of the inflammatory pathways.

The results indicate that, among the tested extracts, the chloroform extract, which is rich in steroids, flavonoids, and saponins, produced >70% inhibition comparable to the standard and exhibited promising anti-inflammatory activity. However, interpretation of the relative efficacy should be supported by appropriate statistical analysis and comparison with the standard anti-inflammatory drug. Further studies involving dose-dependent evaluation, biochemical markers of inflammation, and characterization of the active phytoconstituents would help to elucidate the mechanism responsible for the observed activity.

TABLE 7: Comparison of Anti-Inflammatory Activity Results Obtained for Different Extracts

S. No.	Extract	No. of Animals	Average Body Weight	Dose (mg/kg)	Mean Paw Swelling	Percentage Inhibition
1.	Control	05	230	100	0.316	-
2.	Diclofenac sodium	05	222	05	0.078	75.32%
3.	E1 (Petroleum ether extract)	05	236	100	0.168	46.84%
4.	E2 (Chloroform extract)	05	270	100	0.088	72.15%
5.	E3 (Acetone extract)	05	240	100	0.132	58.23%
6.	E4 (Ethanol extract)	05	230	100	0.158	50.00%

7.	E5 (Water Extract)	05	260	100	0.304	3.80%

Conclusion

In this study, the ash value, extractive value, and chromatographic profile of Oroxylum indicum leaf powder were assessed. Determination of these parameters could aid in the standardization of the plant material. Preliminary phytochemical screening indicated that the chloroform extract contained steroids, flavonoids, and saponins, whereas the ethanol extract revealed alkaloids, carbohydrates, glycosides, and flavonoids. The petroleum ether extract tested positive solely for steroids. The acetone extract demonstrated the presence of flavonoids, and the water extract verified the presence of alkaloids, carbohydrates, saponins, and glycosides. The chloroform extract of leaves of Oroxylum indicum showed maximum anti-inflammatory activity comparable to the standard, followed by acetone, ethanol, petroleum ether, and water extract. However, the interpretation of the relative efficacy should be supported by appropriate statistical analysis and comparison with the standard anti-inflammatory drug. Further studies involving dose-dependent evaluation, biochemical markers of inflammation, and characterization of the active phytoconstituents would help to elucidate the mechanism responsible for the observed activity.

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Conflict Of Interest

The authors have no conflicts of interest.

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