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PHYTOCHEMICAL SCREENING AND IN-VITRO ANTI-INFLAMMATORY ACTIVITY OF AQUEOUS ETHANOLIC EXTRACT OF DRIED LEAVES OF CROTALARIA VERRUCOSA

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ABSTRACT:
The main aim of this study was to determine the pharmacognostic, phytochemical parameters and to determine the anti-inflammatory activity of the aqueous ethanolic extract of Crotalaria verrucosa leaves. The aqueous ethanolic plant extracts of leaves of Crotalaria verrucosa were prepared using the standard maceration method. Then conducted phytochemical screening by various pharmacognostic tests and in-vitro anti-inflammatory activity by protein denaturation method. The preliminary phytochemical analysis revealed that the aq. ethanolic leaf extract of Crotalaria verrucosa were shown positive results towards to carbohydrates, glycosides, phenols, flavonoids, proteins and amino acids and revealed the ethanolic extract showed the positive results towards carbohydrates, glycosides, phenols, proteins and amino acids. We conducted and reported the Inhibitory concentration (IC_{50}) of anti-inflammatory activity of Crotalaria verrucosa leaves hydro -alcoholic extract was found at concentration of 240 µg/mL.
KeyWords: Crotalaria verrucosa, Pharmacognosy, Phytochemistry, Anti-inflammatory activity, denaturation

INTRODUCTION:

India has long been celebrated as the “herbal garden of the world” due to its immense biodiversity and the traditional use of medicinal plants since ancient times. For centuries, humans have depended on plants for nourishment and therapeutic purposes. Among these, Crotalaria verrucosa (commonly known as blue rattlepod) is a leguminous herb belonging to the “Fabaceae” family. It is widely distributed across tropical and subtropical regions, including Bangladesh, Sri Lanka, Southeast Asia, Australia, and Central America. The plant is frequently cultivated as green manure owing to its ability to enhance soil fertility [1].

C. verrucosa thrives in sunny, well-drained soils and grows at elevations of up to 1300 meters, even in saline conditions. Several species of the genus Crotalaria are consumed globally as leafy vegetables or used in dried form as herbal supplements. The plant is valued for its rapid growth, effective ground cover, high biomass production, and weed suppression. Moreover, it maintains a symbiotic association with nitrogen-fixing bacteria that form nodules on its roots, thereby improving soil quality. Traditionally, C. verrucosa leaves have been used in folk medicine for the treatment of rheumatism, skin infections, jaundice, dyspepsia, diarrhoea, fever, dysentery, and leprosy. The leaf juice has also been applied in conditions such as scabies, impetigo, and excessive salivation. Ethno-medicinal records from different regions report its use for various skin disorders, digestive disturbances, and inflammatory conditions [2-5].

Phytochemical studies reveal that C. verrucosa contains a wide range of bioactive compounds, including alkaloids, flavanoids, tannins, glycosides, steroids, polyphenols, and terpenoids. Notably, alkaloids, flavanoids, and tannins from the plant have been reported to possess cytotoxic properties against cancer cell lines, further supporting its pharmacological significance [7].

Fig. 1: Crotalaria verrucosa plant

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MATERIALS & METHODS:

All the chemicals and solvents used in this study were of analytical grade and were procured mainly from Merck. The solution, Biuret reagent, Millon's reagent, Ninhydrin solution, lead acetate solution, Mayer's reagent, Dragendorff's reagent, Benedict's reagent, Fehling's solutions A and B, hydrochloric acid, and ethanol.

Plant Collection and Authentication

Fresh and matured leaves of Crotalaria verrucosa were collected from the botanical garden of Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Guntur. The plant material was authenticated by the Department of Pharmacognosy. The harvested leaves were shade-dried, coarsely powdered, and stored in air-tight containers until further use.

List of Instruments Used:

Weighing balance, Digital PH meter, Ultra Sonicator, Magnetic stirrer, Hot plate, Motor and pestle and UV-visible spectrometer.

Extraction Process

Approximately 20 g of powdered leaves were placed in a conical flask and macerated with an equal proportion of water and ethanol (1:1). The mixture was kept in a dark place for 4–5 days with regular stirring every 24 hours to ensure uniform extraction. After completion of maceration, the mixture was filtered under vacuum, and the filtrate was evaporated on a hot plate to obtain a concentrated crude extract.

Determination of Percentage Yield

Solvent extraction was also performed using different solvents including petroleum ether, benzene, chloroform, ethyl acetate, methanol, and water. The percentage yield of each extract was calculated to determine the efficiency of extraction.

Preliminary Phytochemical Screening

Qualitative phytochemical tests were carried out on the aqueous ethanolic extract to identify major classes of phytoconstituents such as carbohydrates, alkaloids, glycosides, phenols, flavonoids, proteins, and amino acids [7].

Table 1: Percentage yield of solvent extracts

S. No.	Extract	Percentage yield
1	Petroleum ether	1.00 %
2	Benzene	1.00 %
3	Chloroform	2.50 %
4	Ethyl acetate	4.20 %
5	Methanol	4.99 %
6	Water	3.99 %

Fig 2: grinding and maceration using conical flask

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Fig 3: vaccum filtration of extract and grinded powder form of leaves in petridish

Determination of Anti-Inflammatory Activity Aqueous Ethanolic Leaves Extract by Protein Denaturation Method
In-vitro Anti-inflammatory Activity
The anti-inflammatory activity of the extract was assessed using the Protein denaturation method. The reaction mixture (5 mL) contained 0.2 mL of egg albumin, 2.0 mL of phosphate buffer saline (pH 6.4), and 2 mL of different concentrations of the extract (10–50 µg/mL). A control tube containing distilled water instead of the extract was prepared. The mixtures were incubated at 37 ± 2 °C for 15 minutes, followed by heating at 70 °C for 5 minutes. After cooling, absorbance was measured at 660 nm using a UV-visible spectrophotometer.
Ibuprofen was used as the reference standard and treated in the same manner for comparison. The percentage inhibition of protein denaturation was calculated using the formula **[8]**.
The percent inhibition of protein denaturation was calculated by using the following formula.
%Inhibition =100 X (1-A2-A1)
A2 = Absorbance of test sample, A1 = Absorbance of control

RESULTS & DISCUSSION:

The preliminary phytochemical investigation of the aqueous ethanolic extract of Crotalaria verrucosa leaves revealed the presence of several important classes of phytoconstituents. The extract tested positive for carbohydrates, glycosides, phenols, flavonoids, proteins, and amino acids, whereas alkaloids, oils, and waxes were absent. The results were depicted in Table 2. The occurrence of these phytochemicals is significant because they are well-documented for their wide range of biological activities, particularly anti-inflammatory, antioxidant, and antimicrobial effects.
Carbohydrates and glycosides serve as essential bioactive molecules that often play a role in immunomodulation and energy metabolism during inflammatory responses. Phenolic compounds are renowned for their free radical scavenging ability and their capacity to modulate oxidative stress pathways, which are closely linked with inflammation. Flavonoids, one of the key groups identified in this extract, are well-established as natural anti-inflammatory agents due to their ability to inhibit enzymes such as cyclooxygenase (COX) and lipoxygenase (LOX), both of which are central mediators in the biosynthesis of prostaglandins and leukotrienes. Proteins and amino acids in the extract may not directly contribute to pharmacological activity but often act as precursors for bioactive peptides or interact synergistically with secondary metabolites to enhance therapeutic efficacy.
The absence of alkaloids, in this case, is noteworthy because many species of Crotalaria are known to contain pyrrolizidine alkaloids, which are hepatotoxic. Therefore, the lack of detectable alkaloids in the studied extract may indicate a safer pharmacological profile.
Results of Anti-inflammatory Activity-Protein Denaturation Assay
The in-vitro anti-inflammatory activity of the aqueous Ethanolic extract was assessed using the protein denaturation method, a simple yet reliable technique to evaluate the ability of a compound to stabilize proteins against heat-induced denaturation. Protein denaturation is considered one of the key events in the development of inflammation, leading to the production of auto-antigens associated with conditions such as rheumatoid arthritis. Therefore, agents that inhibit protein denaturation are often regarded as having potential anti-inflammatory activity.
The extract demonstrated a clear dose-dependent inhibition of protein denaturation. At lower concentrations, the activity was modest but increased progressively with higher concentrations. At the highest tested concentration, the inhibition rate was comparable to the standard drug, ibuprofen, indicating the strong potential of C. verrucosa as a natural anti-inflammatory agent.

Thus the anti-inflammatory effect of C. verrucosa can be explained by the presence of multiple classes of phytoconstituents working through complementary mechanisms like the presence of flavanoid compounds can inhibit enzymes like COX and LOX, reducing the synthesis of pro-inflammatory prostaglandins and leukotrienes. Additionally, flavanoids stabilize lysosomal membranes, preventing the release of proteolytic enzymes during inflammation. Similarly the presence of Phenolic Compounds, which act as antioxidants by neutralizing reactive oxygen species (ROS).
Therefore the results of this study demonstrate that the aqueous Ethanolic extract of C. verrucosa leaves is rich in phytochemicals with known pharmacological activities. The extract exhibited a dose-dependent anti-inflammatory effect in the protein denaturation assay, with an IC_{50} value of 240 µg/mL and 256 µg/mL for standard (Ibuprofen). The activity is most likely due to the synergistic action of flavanoids, phenolic compounds, and glycosides, which inhibit protein denaturation and prevent the release of inflammatory mediators. These findings validate the ethno medicinal use of *C. verrucosa* for the treatment of inflammatory conditions and open avenues for further research aimed at drug discovery and development.

Table 2: Preliminary Phytochemical Screening of Aqueous Ethanolic Leaf Extract of Crotalaria verrucosa.

S.No	Tests	Leaves
1	Carbohydrates	+
2	Flavonoids	-
3	Alkaloids	-
4	Glycosides	+
5	Proteins	+
6	Phenols	+
7	Amino acids	+
8	Oils and Waxes	-

The preliminary phytochemical analysis reveals that the hydro alcoholic leaf extract of Crotalaria verrucosa showed positive results towards carbohydrates, glycosides, phenol, proteins, amino acids and negative results towards the Alkaloids and flavonoids.

Table 3: Preliminary Phytochemical Screening of Aqueous Leaf Extract of Crotalaria verrucosa.

S.No	Tests	Leaves
1	Carbohydrates	+
2	Flavonoids	-
3	Alkaloids	-
4	Glycosides	+
5	Proteins	+
6	Phenols	+
7	Amino acids	+

Table 4: Determination of anti-inflammatory activity of Hydro alcoholic extract of Crotalaria Verrucosa Leaves by Protein Denaturation Method

S.No	Concentration (µg/ml)	Inhibition Concentration (%)	Concentration (µg/ml)	Inhibition Concentration (%)
1	200	47.7±0.4	100	17.3±0.6
2	400	55.4±1.5	200	32.5±2.3
3	600	61.5±2.6	300	43.7±3.8
4	800	65.8±3.2	400	78.2±4.1
5	1000	68.2±3.9	500	100.4±5.2

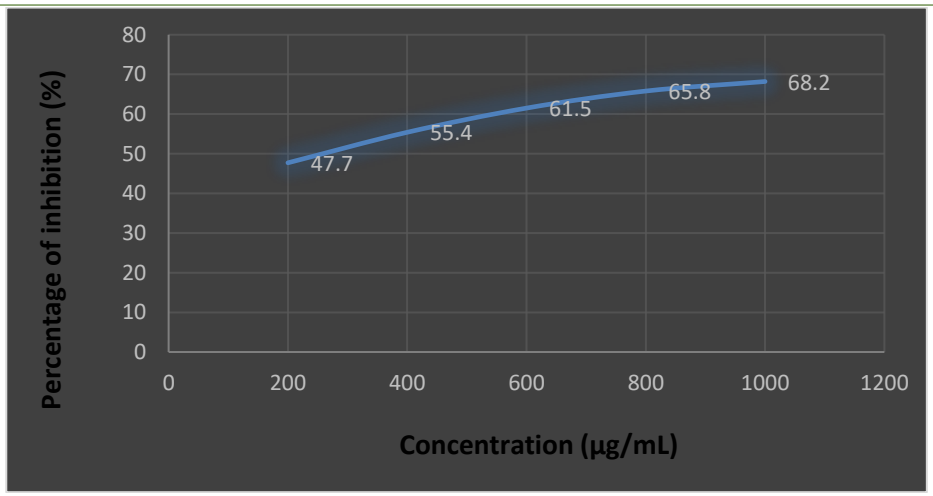

Fig 5: Anti-inflammatory activity of hydro alcoholic extract of leaves by protein denaturation method

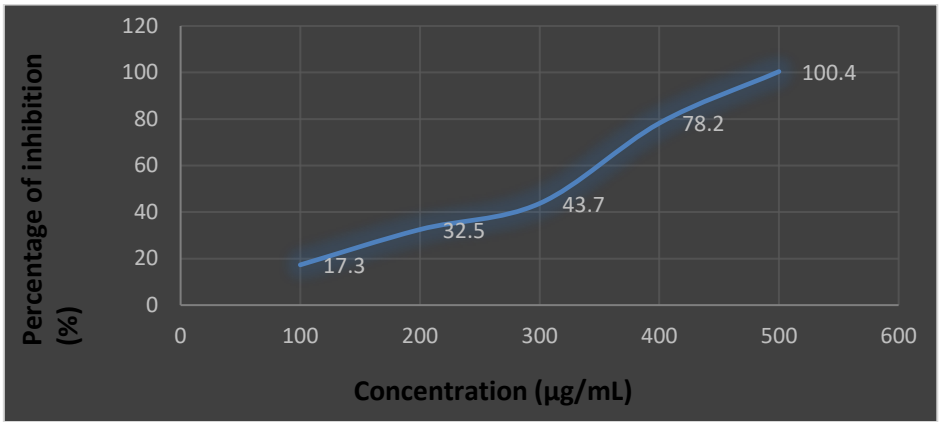

Fig 6: Anti-inflammatory activity of Ibuprofen (Standard) by protein denaturation method

CONCLUSION

The present study explored the pharmacognostic, phytochemical, and in-vitro anti-inflammatory properties of the aqueous Ethanolic extract of Crotalaria verrucosa leaves. The research was designed with the aim of providing scientific validation for the traditional use of this plant in inflammatory disorders. The findings contribute to the growing body of evidence that medicinal plants, particularly those rich in secondary metabolites, can play a vital role in modern drug discovery and development.

The phytochemical screening of the extract confirmed the presence of carbohydrates, glycosides, phenolic compounds, flavanoids, proteins, and amino acids. Each of these groups is known to possess biological activity, especially in the context of inflammation and oxidative stress. Flavanoids and phenolics, in particular, are well-documented for their ability to inhibit pro-inflammatory enzymes and reduce oxidative damage, while glycosides are often associated with immunomodulatory effects. Together, these phytochemicals form a synergistic matrix that likely accounts for the observed pharmacological activity.

The in-vitro evaluation of anti-inflammatory activity using the protein denaturation assay revealed that the extract displayed significant, dose-dependent inhibition of protein denaturation. The calculated IC_{50} value of 240µg/mL, although modest compared with synthetic standards like ibuprofen, is considered noteworthy for a crude plant extract.

Future research should focus on fractionation and isolation of individual bioactive constituents from C. verrucosa. Such an approach may lead to the discovery of lead molecules with stronger activity at lower concentrations. Structural elucidation of these compounds, followed by molecular docking and mechanistic studies, could provide valuable insights into their interaction with key inflammatory mediators. Moreover, clinical studies will ultimately be necessary to confirm the efficacy and safety of C. verrucosa extracts in human populations.

In conclusion, the present study demonstrates that the aqueous Ethanolic extract of Crotalaria verrucosa leaves exhibits considerable anti-inflammatory potential, supported by the presence of phytochemicals with known therapeutic activity. These results not only validate the ethno medicinal use of the plant but also highlight its promise as a candidate for natural anti-inflammatory drug development. With further in-depth studies focusing on mechanism, safety, and clinical efficacy, C. verrucosa could emerge as a valuable addition to the arsenal of plant-based medicines aimed at combating inflammatory diseases.

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