



WORLD JOURNAL OF CURRENT MEDICAL AND PHARMACEUTICAL RESEARCH

www.wjcmpr.com

ISSN: 2582-0222

Phytochemical screening & *in-vitro* evaluation of anti-inflammatory activity of *clerodendrum indicum* roots

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Abstract

This study aims to extract the phytochemical constituents and *in-vitro* evaluation of anti-inflammatory activity of roots of *clerodendrum indicum*. *Clerodendrum indicum* (L.) kuntz. Commonly known as bharangi or chingari, belongs to the family verbenaceae. The plant is expectorant, stomachic, Anti-bronchitis, Anti-nociceptive, Anti-microbial, Anti-diarrheal, anti-oxidant and anti-cancer. Preliminary phytochemical screening revealed the presence of flavonoids, steroids, steroid glycosides, terpenoids etc. This study has taken with the Anti-inflammatory activity and preliminary phytochemical screening on the *clerodendrum indicum*. In the present study, qualitative study of the phytochemicals present in the sample was determined, Anti-inflammatory activity were carried out. On the premise of the prevailing study, the outcomes eventually concluded that the ethanolic extract of *clerodendrum indicum* own anti inflammatory pastime. This evaluation offers an concept that the compound of the plant *clerodendrum indicum* may be used as lead compound for designing robust pills which may be used for remedy of diverse diseases.

Article History:



Received: 13.09.2021

Revised: 05.10.2021

Accepted: 22.11.2021

Keywords:

clerodendrum indicum, Phytochemical screening, Anti-inflammatory activity, Methanolic extract, Verbenaceae

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DOI: <https://doi.org/10.37022/wjcmpr.v3i6.201>

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Introduction

Traditional medicine has a long history in india. Traditional medicine is an essential component of health treatment. The majority of people in poor nations still rely on indigenous traditional medicines to meet their basic health care needs. India's materia medica has a wealth of information on folklore traditions and traditional characteristics of therapeutically significant natural products. Folklore living in the nooks and crannies of every country are now acclimated to using their plant resources. They have established ethnic medical systems. Some of them have emerged as a result of extensive experience [1]. India, china, and a number of other countries have a long history Herbal medicine has a long history. More than 800 herbal cures can be found in the recorded records of Ayurveda, India's traditional system of medicine. The Charaka Samhita and the Sushruta Samhita are two treasure troves of plant-based drug knowledge that are still held in high regard around the world [2].

Plant introduction

It is a tiny shrub that is classified as a "environmental weed," "naturalised," and "weed" [3] in the global weed compendium. The species is thought to be endemic to southern China, India, Malaysia, and possibly the Phillipines [4]. It was introduced as an ornamental in temperate and tropical regions

internationally, escaped cultivation in 1914, and is now established in the neotropics Fig 01.



Fig 01: clerodendrum indicum plant

Seeds, rooted cuttings, and suckers were used to proliferate the plant, although it did not appear to be as invasive as other members of the *clerodendrum* genus and did not pose a substantial danger to native vegetation. Given its increasingly vast dispersion outside of its natural region, quick growth rate, and ability to reproduce both by seeds and vegetatively [5], monitoring and future appraisal are advised.

It is a tiny shrub that can reach a height of 3 metres when developed from an aggressively suckering, stoloniferous root source. Typically, the stem is fairly straight and unbranched. The herb is gathered in the wild for local medicinal purposes [6]. Tube flower, sky rocket, bowing Lady, bharangi, hunjuki, bh argi, and other common names for *clerodendrum indicum* inclu

de tube flower, sky rocket, bowing Lady, bharangi, hunjuki, bharangi, and others. The leaves of *clerodendrum indicum* are used as a bitter tonic and vermifuge, and the dried leaves are smoked like cigarettes to relieve asthma [7].

In Ayurvedic medicine, the plant juice is applied externally to treat skin problems. asthma, cough, and other pulmonary symptoms can be treated with the roots in combination with ginger. Syphilitic rheumatism is treated using a resin derived from the plant [8].

The many activities seen on the *clerodendrum indicum* [9-11]

- Anti nociceptive Activity
- Anti Diarrheal Activity
- Anti Microbial Activity
- Anti oxidant Activity
- Anti cancer properties
- Cytotoxic Activity
- Anti-bronchitis

Materials and Methods

All the chemicals and solvents used were obtained from merc company Bangalore, SDFCL Laboratories Mumbai. Glassware was also procured from Titan Biotech.

Plant collection and authentication

The fresh roots of *clerodendrum indicum* were collected during the months of November-December from the S.V Botanical Garden.

The plant material was taxonomically identified and authenticated by Dr.Madhavashetty, Department of Botany, Sri Venkateshwara University, Tirupathi.

The plant voucher number-0542

Preparation of Phytochemical Extract of Plant

SOXHLET (HOT CONTINUOUS EXTRACTION)

In this method, the finely floor crude drug is positioned in a porous bag or "thimble" made from sturdy clear out paper, that's positioned in chamber of the Soxhlet apparatus. The extracting solvent in flask A is heated, and its vapours condense in condenser. The condensed extractant drips into the thimble containing the crude drug, and extracts it with the aid of using contact. When the extent of liquid in chamber rises to the pinnacle of siphon tube, the liquid contents of chamber siphon into flask. This manner is non-stop and is accomplished till a drop of solvent from the siphon tube does no longer depart residue whilst evaporated. The benefit of this method, as compared to formerly defined methods, is that massive quantities of drug may be extracted with a far smaller amount of solvent. This results high-quality economic system in phrases of time, strength and therefore monetary inputs. At small scale, it's far hired as a batch manner only, however it will become an awful lot extra reasonably-priced and feasible whilst transformed right into a non-stop extraction system on medium or massive scale[11].

Preparation of Extraction

The plant fabric changed into dried beneathneath coloration at room temperature for approximately 10 days.the dried plant samples have been powdered via way of means of mechanical grinder and sieved to present particle length 50 to one hundred fifty mm. the powder changed into saved in polythene baggage at room temperature earlier than extraction powder (100g) changed into crammed withinside the thimble and extracted successively with 70% methanol in soxhlet extractor.

Extraction changed into accomplished for sixteen hrs until the coloration of the solvents on the closing siphoning time returns coloration Solvents have been evaporated beneathneath decreased strain the usage of rotary evaporator apparatus.

In-Vitro Anti-Inflammatory Assay

Inhibition of Protein Denaturation Using Egg Albumin[12]

The response mixture (5 ml) consisted of 0.2 ml of egg albumin (from clean hen's egg), 2.8 ml of phosphate-buffered saline (PBS, pH 6.4), and a couple of ml of various concentrations of Methanolic extract of *clerodendrum indicum* in order that very last concentrations emerge as 100, 200, 300, 400, and 500 µg/ml. A comparable extent of double distilled water served as control. Then, the combos have been incubated at 37 ± 2°C in a BOD incubator for 15 min after which heated at 70°C for five min. After cooling, their absorbance become measured at 660 nm. Diclofenac sodium become used as a reference trendy drug. The percentage inhibition of protein denaturation was calculated using the following formula:

$$\text{Percentage inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

Results and Discussion

Finally extracts allowed to air in Petri dish till complete dryness and the percentage yield was calculated as follow:

Table 01: percentage of yield

Sample	Weight of sample in gms	Weight of the extract	% yield
<i>Clerodendrum indicum</i> methanol extract	100 gm	20 gm	20 %

Phytochemical Screening of Methanolic Extract of *Clerodendrum Indicum*

Table 02: phytochemical screening results

S. No	Test for	Methanolic extract of <i>clerodendrum Indicum</i>
1	Carbohydrates	-
2	Steroids	+
3	Cardiac glycosides	-
4	Steroid glycosides	+
5	Coumarins	-
6	Flavonoids	+
7	Alkaloids	-
8	Tannins	-
9	Terpenoids	+
10	Volatile oils	-

Thin layer chromatography [13]

Rf values of flavonoid compounds identified and their colours on TLC chromatography under the UV light

Table 03: Rf values

Flavonoids	Rf value	Color under UV 365 nm
Orientin	0.6	Light yellow
Hyperoside	0.7	Dark orange

Table 04: In vitro anti-inflammatory effect of clerodendrum indicum evaluated by protein denaturation method

S.No	Composition	Concentration (µg/ml)	Absorbance at 660 nm (mean ± SD)	Inhibition of Protein denaturation
1	control	-	0.337 ± 0.0008	-
2	Extract	100	0.216 ± 0.001	35.9
		200	0.202 ± 0.002	40.0
		300	0.173 ± 0.001	48.6
		400	0.147 ± 0.001	56.3
		500	0.113 ± 0.001	66.4
		3	Diclofenac sodium	100

Values are mean ± SD,

The inhibitory impact of various concentrations of methanolic extract of clerodendrum indicum on protein denaturation as proven in desk form. Extract (100-500 µg/ml) and diclofenac sodium (100-two hundred µg/ml) confirmed enormous inhibition of denaturation of egg albumin in awareness structured manner. Effect on protein denaturation make contributions to the in-vitro anti-inflammatory interest of the clerodendrum indicum root extract utilized in our study.

In the existing study, dried powder roots of clerodendrum indicum had been subjected to extraction used methanol as solvent with the aid of using soxhlet procedure. Some a part of extract became reserved for initial phytochemical research and relaxation became applied for phytochemical screening and pharmacological pastime. The Preliminary Phyto-chemical research confirmed presence of flavonoids, steroids, steroid glycosides, terpinoids compounds. The flavonoid compound being the important Phyto-chemical constituent became remoted methanolic extract of roots and subjected to qualitative TLC analysis. Isolate the ingredients the usage of chromatographic method (TLC) with the aid of using the usage of cellular section withinside the ratio of 100:11:11:27 (ethyl acetate : acetic acid : formic acid : water). Investigated in-vitro assessment of anti inflammatory pastime of clerodendrum indicum plant roots. Protein Denaturation is a procedure wherein proteins lose their tertiary shape and secondary shape with the aid of using utility of outside pressure or compound,

including sturdy acid or base, a focused inorganic salt, an natural solvent or heat. Most organic proteins lose their organic characteristic whilst denatured. Denaturation of proteins is a properly documented reason of inflammation. The increments in absorbance of take a look at samples with admire to manipulate indicated stabilization of protein i.e., inhibition of heat-caused protein (albumin) denaturation with the aid of using clerodendrum indicum and reference drug diclofenac sodium. From the proportion inhibition of protein denaturation values it turns into obvious that clerodendrum indicum became extra lively than diclofenac sodium, being powerful in decrease concentrations. In the existing study, the in-vitro anti inflammatory pastime of clerodendrum indicum may be attributed to its flavonoid content. The impact can be because of the synergistic impact instead of unmarried constituent. clerodendrum indicum confirmed most inhibition of 66.4% on the awareness of 500 µg/ml. Diclofenac sodium, a widespread anti inflammatory drug confirmed the most inhibition of 59.3% on the awareness of 100µg/ml as compared with control.

Conclusion

On the idea of the prevailing study, the effects in the end concluded that the ethanolic extract of clerodendrum indicum own anti inflammatory pastime. This pastime can be because of the sturdy incidence of compounds along with alkaloids, flavonoids, tannins, steroids, and phenols. This evaluation offers an concept that the compound of the plant clerodendrum indicum may be used as lead compound for designing mighty pills which may be used for remedy of numerous diseases. However those research aren't enough to assert and as a result numerous different pharmacological, phytochemical and bio analytical research observed with the aid of using observational research in people are to be performed for you to aid conventional significance of the said pastime..

Acknowledgment

Authors are thankful to the IBST and SRMU for providing us this opportunity to carry out the work.

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