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RESEARCH

Phytochemical Screening and Anti-Arthritic Activity of Methanolic Leaves Extract of *Phyllanthus Niruri*

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ABSTRACT

The study was based on evaluation of phytochemicals and anti-arthritic potential of methanolic leaves extract of *Phyllanthus niruri*. Fresh leaves of *Phyllanthus niruri* were obtained from the Unnao region. These were identified and authenticated by a botanist at BSI, Prayagraj (ref. 2024-25/543). Leaves were washed and dried in shade. The powder was weighed and soaked into methanol (95%) for 15 days with gradual stirrings. The obtained slurry of mixture was dried under partial vacuum using a rotary evaporator. Extraction the plant was done using methanol (95%) through maceration. Evaluation of the anti-arthritis activity of the methanolic herbal extract was performed through Inhibition of protein denaturation using bovine serum albumin and Inhibition of protein denaturation using egg albumin. The results showed that the methanolic leaves extract of *P. niruri* demonstrated the % inhibitionddddddddd of protein denaturation using BSA as 87.2±0.1 % and 92.5±0.4 % at the conc. of 400 µg/ml and 800 µg/ml respectively. It concluded that the anti-arthritic activity was found in methanolic leaves extract of *P. niruri*. On the other hand, a dose-dependent biological response was discovered. Furthermore, in both models, the extract's aqueous fraction demonstrated a higher percent suppression of protein denaturation. The mechanism of action by which methanolic leaves extract of *P. niruri* treat and stop the progression of rheumatoid arthritis will be assessed by fellow researchers.

Keywords: *Phyllanthus niruri,* egg albumin, anti-arthritic, protein denaturation.

INTRODUCTION

It can impact places other than the joints and is an inflammatory kind of arthritis [1]. It is mostly brought on by environmental causes, including smoking cigarettes, and genetic predisposition [2][3]. Typically, it affects small peripheral joints first, but if addressed, it can spread to proximal joints and become symmetric [4]. When inflammation erodes cartilage and pushes bone into the joint socket, degeneration of the joints results. As per Global Burden of Disease 2010, the prevalence of RA is approximately 0.24% worldwide [5]. Based on epidemiological data, women are more likely than men to develop RA [6].

Phyllanthus niruri

Phyllanthus niruri is a herb that reaches a height of 30 to 60 cm. It is glabrous, and the stem frequently branches at the base. The leaves are elliptic, oblong, obtuse, sessile, distichous, and many. There are many small, green, subsessile, closely spaced, oval, ablong, obtuse leaves with stipules and a short petiole. The leaves are arranged alternately on either side of the stem. The flowers are axillary, tiny, yellowish, and abundant. These blooms are unisexual and monoecious; the females are solitary in nature, while the males have one to three sessile stamens. The fruit is a tiny, flattened, globose capsule that is smooth and has a

diameter of 2-3 mm. Its horizontal branches are 30 to 60 cm in height and 1 to 2.5 mm in width. It is big and somewhat branching [7].

Taxonomy [8]

Kingdom- Plantae

Order- Euphorbiales

Family- Euphorbiaceae

Genus- Phyllanthus

Species- *niruri*

Table 1. Phytoconstituents [9]

Category	Phytoconstituents
Alkaloid	4-Methoxy-nor-securinine, nirurin, ent-norsecurin
Benzenoid	Gallic acid, Corilagin
Coumarin	Ellagic acid, ethyl brevifolin carboxylate
Flavonoid	Quercetin, rutin, astragalin, quercitrin, isoquercitrin, kaempferol-4-rhamnopyranoside,eridictyol-7-rhamnopyranoside, fisetin-4-O-glucoside, nirurin
Lignin	Phyllanthin, hypophyllanthin, niranthin, nirtetralin, phyltetralin, hinokinin, isolintetralin
Lipid	Ricinoleic acid
Phytallate	Phyllester
Sterol	Estradiol, ß-sitosterol, isopropyl-24-cholesterol
Tannin	Geranin
Triterpene	upeol acetate, lupeol, 3,7,11,15,19,23-hexamethyl-2Z,6Z,10Z,14E,18E, 22E-tetracoshenen1-ol, phyllanthenol, phyllanthenone, phyllantheol.

MATERIALS AND METHODOLOGY

Experimental Requirements

Fresh leaves of *Phyllanthus niruri,* bovine serum albumin, egg albumin, distilled water, indomethacin, diclofenac, paraffin, and methanol.
Digital balance, round bottom flask, condenser, clinical thermometer, and digital pH meter.

Collection, Authentication and Extraction of plant

Fresh leaves of *Phyllanthus niruri* were obtained from the Unnao region. These were identified and authenticated by a botanist at BSI, Prayagraj (ref. 2024-25/543). These were cleaned to remove dust and let to dry in the shade or at room temperature. These were subsequently ground into coarse and then fine powders. After being weighed, the powder was steeped in 95% methanol for 15 days while being stirred gradually. The obtained slurry of mixture was dried under partial vacuum using a rotary evaporator.

Phytochemical Screening [10][11]

Alkaloids

Before being filtered, each extract is dissolved in diluted HCl.

Mayer's Test: The filtrate treated with Mayer's reagent. The production of a yellow precipitate shows that there are alkaloids present.

Wagner's Test: The filtrates treated with Wagner's reagent. When a brown or reddish precipitate appears, it confirms the presence of alkaloids.

Glycosides

Fehling's solutions A & B are heated for one minute with distilled water. Eight drops of plant extract were added to this clear blue solution. After that, it is cooked in a water bath for five minutes while mixed with one milliliter of Fehling's solution. The presence of glycosides is indicated by brick-red precipitation.

Saponins

Herbal extract mixed with 10ml water and agitated quickly to make a stable, long-lasting foam. Foam shows that saponins are there.

Tannins

Ferric chloride test: In a test tube, 20ml of water is used to boil 0.5g of the dried powdered sample, which is subsequently filtered. After adding a few drops of 0.1% FeCl3, the color is tested to see if it is brownish green-black.

Lead acetate test: Mix 2g extract with 2ml distilled water. The mixture is shaken well after 0.01g of lead acetate is added. When white turbidity and precipitate form, tannins are present.

Flavonoids

NaOH test: A tiny quantity of extract is treated with aqueous NaOH and HCl, and the development of a yellow-orange hue is monitored.

H_2SO_4 test: A part of the extract is treated with conc. H2SO4, and the color change to orange is watched.

Terpenoids

Mix 2.0 ml of chloroform with 5 ml of extract in water. Then, add the mixture to the water route, let it evaporate, and boil it with 3 ml of concentrated H2SO4. When the terpenoids grew, a gray color appeared.

Steroids

The 5g extract is mixed with 2ml of chloroform and conc. H2SO4. The red color that appeared in the bottom chloroform layer shows that steroids are present.

Estimation of reducing sugars and carbohydrates

Molisch's test

Put 2–3 ml of extract of different solvents in a test tube and add a few drops of α-naphthol solution in alcohol. Then, shake the test tube and add conc. H2SO4 from the sides. violet ring where two liquids touch.

Fehling's test

It is used to find sugars that are going down. Dissolve 34.66 grams of copper sulfate in 500ml distilled water (solution A). To make Solution B, mix 50g sodium hydroxide and 17.3 grams of potassium sodium tartrate in 50ml distilled water. Before using, mix two solutions in the same amount. You should boil Fehling's A and B solutions together for one minute. You should add equal amounts of the test solution. Put in a pot of boiling water and cook for five to ten minutes. At first, it was yellow, and then it turned brick red.

Evaluation of anti-arthritic activity

- **Inhibition of protein denaturation using bovine serum albumin (BSA)**

The reaction mixture (0.5ml) contains 0.45ml of BSA (5% aqueous solution) and 0.05 ml of various concentrations of methanolic leaves extract of *Phyllanthus niruri* and indomethacin (reference medication) (12.5, 25, 50, 100, 200, 400, & 800µg/ml), respectively. Using 1N HCl, the solution was adjusted to pH 6.3. The samples were heated to 57°C for 30 minutes after being incubated for 20 min at 37°C. Next, 2.5 ml of phosphate buffer was added, and a spectrophotometer was used to detect absorbance at 660 nm. The methanolic leaves extract of *Phyllanthus niruri* was replaced with 0.05 ml of distilled water for the test control, whereas BSA was absent from the product control [12].

Using the following formula, it was calculated:

$$\% \text{ inhibition} = [\text{Absorption control} - \text{Absorption test}/\text{Absorption Control}] \times 100$$

- **Inhibition of protein denaturation using egg albumin**

Diclofenac sodium was added to the reaction mixture (5 ml) at different concentrations (12.5, 25, 50, 100, 200, 400, and 800µg/ml), egg albumin (0.2 ml), phosphate buffered saline, 2.8 ml, and 2 ml of methanolic leaves extract of *Phyllanthus niruri* crude extract and fractions, respectively. A control group of the same volume of double-distilled water was used. The mixtures were heated to 70 °C for five minutes after being incubated for fifteen minutes at 37 ± 2 °C in a biochemical oxygen demand (BOD) incubator. At 660 nm, their absorbance was measured [12].

Formula:

$$\% \text{ inhibition} = [\text{Absorption control} - \text{Absorption test}/\text{Absorption Control}] \times 100$$

RESULTS AND DISCUSSION

Percentage yield

The percentage yield was obtained for methanolic leaves extract of *Phyllanthus niruri* as 63.28 %.

Phytochemical screening

Table 2. Phytochemicals of methanolic leaves extract of *Phyllanthus niruri*

Phytoconstituents	Methanolic extract
Alkaloids	++
Glycosides	+++
Flavonoids	++
Tannins	+
Saponins	+++
Terpenoids	++
Steroids	+
Phenols	+
Coumarins	++
Anthocyanin	+++

Absent (-), Moderate (++), Abundance (+++)

Evaluation of anti-arthritic activity

- **Inhibition (%) of protein denaturation through bovine serum albumin**

Methanolic leaves extract of *P. niruri* demonstrated the % inhibition of protein denaturation using BSA as 87.2±0.1 % and 92.5±0.4 % at the conc. of 400µg/ml & 800µg/ml respectively.

Table 3. Determination of inhibition of protein denaturation through bovine serum albumin of methanolic leaves extract of *P. niruri*

Treatment	Inhibition of protein denaturation [Conc. (µg/ml)]						
	12.5	25	50	100	200	400	800
Methanolic leaves extract of *P. niruri*	63.2±0.6	71.1±0.3	75.1±0.3	79.3±0.5	84.1±0.3	87.2±0.1	92.5±0.4

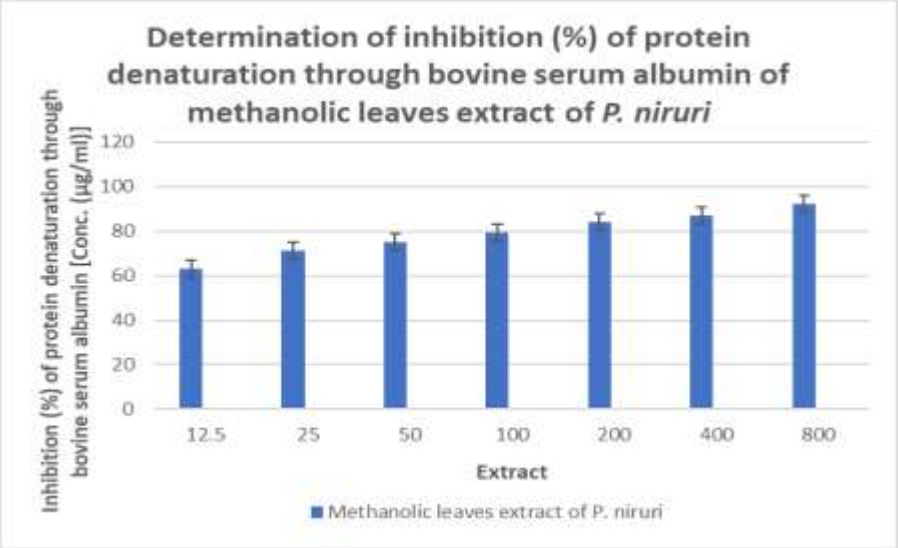

Fig 1. Determination of inhibition (%) of protein denaturation through bovine serum albumin of methanolic leaves extract of *P. niruri*

- **Inhibition (%) of protein denaturation through egg albumin**

Methanolic leaves extract of *P. niruri* demonstrated the % inhibition of protein denaturation using egg albumin as 87.2±0.4 % and 91.4±0.3 % at the conc. of 400µg/ml & 800µg/ml respectively.

Table 4. Inhibition of protein denaturation through egg albumin of methanolic leaves extract of *P. niruri*

Treatment	Inhibition (%) of protein denaturation [Conc. (µg/ml)]						
	12.5	25	50	100	200	400	800
Methanolic leaves extract of *P. niruri*	44.6±0.3	61.4±0.2	65.2±0.7	73.2±0.2	76.5±0.6	87.2±0.4	91.4±0.3

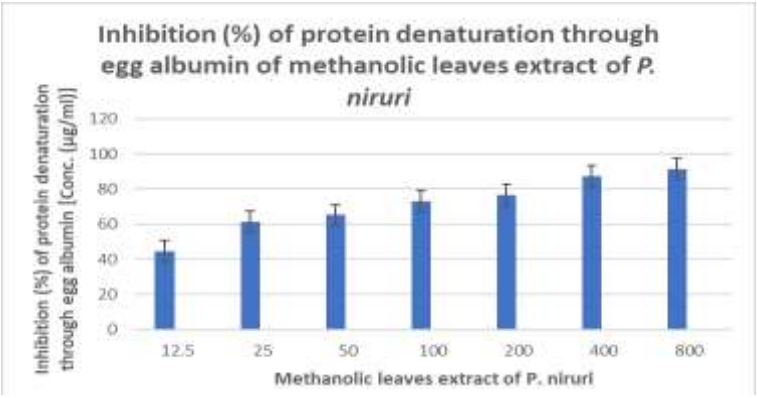

Fig 2. Inhibition (%) of protein denaturation through egg albumin of methanolic leaves extract of *P. niruri*

It is possible to extrapolate the results of the in vitro antiarthritic membrane stabilization approach to the impact of B. calliobotrys that prevent muscular atrophy. Similarly, hypermetabolism mediated by cytokines is considered to be the cause of rheumatoid cachexia. Previous studies have also suggested that the gut of rats has a decrease in the absorption of 14C-glucose and 14C-leucine when it is inflamed, and that this reduction can be restored by anti-inflammatory medications. The current study's findings indicate that the aqueous fractions of B, n-bustanol fraction, and methanolic extract. Rats with arthritis benefit greatly from

calliobotrys' protective effect on body weight. Thus, the antiarthritic action of B has been established by histological studies. Calliobotrys by reducing the inflammatory response, potentially as a result of its inhibition of the cyclooxygenase enzyme and pro-inflammatory cytokines. Numerous plants in the genera Berberis and Coptis contain the pharmacologically powerful isoquinoline alkaloid berberine. In a number of autoimmune disorders, berberine has been demonstrated to have immunosuppressive and anti-inflammatory properties through the suppression of Th17 and dendritic cell responses. Furthermore, by blocking the most common variables associated with arthritis, berberine also helps to reduce joint inflammation and severe pain [13].

In results, the methanolic leaves extract of *P. niruri* demonstrated the % inhibition of protein denaturation using BSA as 87.2±0.1 % and 92.5±0.4 % at the conc. of 400 µg/ml and 800 µg/ml respectively. Nonetheless, the methanolic leaves extract of *P. niruri* demonstrated the percent inhibition of protein denaturation using egg albumin as 87.2±0.4 % and 91.4±0.3 % at the conc. of 400µg/ml and 800µg/ml respectively.

CONCLUSION

Rheumatoid arthritis can cause permanent disability and joint degeneration. Early identification and treatment are essential to preventing irreversible damage and the loss of essential bodily functions. The anti-arthritic activity of methanolic leaves extract of *P. niruri* was concluded. On the other hand, a dose-dependent biological response was discovered. Furthermore, in both models, the extract's aqueous fraction demonstrated a higher percent suppression of protein denaturation.

It might be useful in the management of RA, that affects a significant portion of the global population. It will be easily accessible to humans and cost-effective. The mechanism of action by which methanolic leaves extract of *P. niruri* treat and stop the progression of rheumatoid arthritis will be assessed by fellow researchers.

CONFLICT OF INTEREST

Authors declare for none conflict of interest.

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