

In-vitro* Anthelmintic and Anti-Arthritic activity of Alcoholic extract of *Nymphoides macrospermum vasud

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Abstract: *Nymphoides macrospermum vasud* (granthika tagara) is incorporated in various preparations used for the treatment of diseases like Tuberculosis, jaundice, mental disorders and etc., This study is aimed at evaluating the *in-vitro* anthelmintic activity of alcoholic extract of *Nymphoids macrospermum vasud* (*Menyanthaceae*) against Indian earth worm *Pheritima postuma*. The extraction process was carried out using alcohol as solvent with soxhlet apparatus and the alcohol was evaporated. Various concentrations of dried extract such as (250, 500 and 1000µg/ml of water) was tested and results were expressed in terms of time for paralysis (1-2 minutes) and time for death of worms (2-4minutes), taking Metrinidazole (50µg/ml) as reference standard, and distilled water as control group. From the results of this study, it was found that the plant roots of *Nymphoids macrospermum vasud* (alcoholic extracts) possess very good anthelmintic activity.

Key words: Anthelmintic activity, tannins, Metrinidazole, Diclofenac Sodium.

INTRODUCTION

Plant medicines helps to develop effective drugs with less toxicity, which have been used from ancient days till today for treating infectious disease in animals and human beings¹. In endemic areas the parasite infection in animals causes reduction in weight, lowered meat and milk production and even leads to morbidity. Due to resistance in the gastro intestinal tract (GIT) absorption with currently available drugs, there is a need for new naturally obtained non-toxic drugs to treat helminthiasis. Tagara (*Valeriana jatamansi jones*, Valerianaceae family) is an important ayurvedic drug employed in several preparations used in the treatment of various diseases. In south India, a formulation is used in the name Granthika tagara (Tamil), botanically

identified as *Nymphoides macrospermum* (*Menyanthaceae*). It is employed in the treatment of various illness such as epilepsy, anemia, jaundice, tuberculosis, mental disorders, fevers, cough and asthmatic conditions and also as a general and brain tonic. The accepted botanical source of Tagara is *Valeriana jatamansi Jones* (*Valerianaceae*). The genus *Nymphoids* consists of about 20 species, of which five are found in India; they are aquatic herbs, floating or creeping with white or yellow flowers. *N. macrospermum* is recorded as a new taxon from south India. Recently a report shows that the plant has Anti-sedative activity² and Antimicrobial activity³

MATERIALS AND METHODS

MATERIALS

Plant material collection and Identification

The root and rhizome constituting the drug of commerce of *N. macrospermum* was procured from the crude drug market at Coimbatore, Tamil Nadu, India, during March 2010. The characters found in the market sample were pharmaco-botanically analyzed, identified as *N. macrospermum*, and authenticated by Dr. N. Ravichandran, CARISM, SASTRA University, Thanjavur, Tamil Nadu.

Extraction

Shade dried roots and rhizomes (75gms) were powdered by milling and sieved using 40 mesh sieves for uniform size distribution. Then the powdered plant material was treated with petroleum ether (40:60) to remove the fats and unnecessary compounds for by using soxhlet extraction procedure done for 48 hrs. Then the wet content was allowed to dry over night to remove the petroleum ether. The dried powder was treated with ethanol 99.99% (absolute ethanol) to extract the active components using the Soxhlet apparatus, the process was carried out again for 48 hrs. The extract was collected and preserved in desiccator to prevent any contamination and moisture absorption and used to carry out antimicrobial activity.

Worm Collection and Authentication

Healthy adult Indian earthworms *Pheritima posthuma* (due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings) were used in the study. All the earth worms were of approximately equal size (6 cm). They were collected from local moist place, washed and kept in water and authenticated.

METHODS

Anthelmintic activity

Anthelmintic activity of the extract was evaluated by exposing the adult *pheritima posthuma* to different

concentration of aqueous solution of *N. macrospermum* species (Table 1). The activity was performed according to the method of Vedha Hari *et.al* with slight modification, on adult Indian earth worm *pheritima posthuma* as it has anatomical and physiological resemblance with the intestinal earthworm parasites of human beings. All the earthworms were washed in normal saline solution before they were released into respective formulation. In each Petri dish three equal size worms were placed and various concentrations of aqueous solutions of the extract (test drug) and Metronidazole (10mg/ml) as reference drug and distilled water as control were used. Observation was made for the time taken for paralysis and death of individual worms. For all the above samples triplets were performed and tabulated⁴.

***In vitro* anti-arthritis activity by inhibition method⁵**

In this method the solutions used are: the test solution of 0.5ml consisting of test sample solution of 0.05ml (250mcg/ml) and bovine serum albumin of 0.45ml (5%w/v aqueous solution), the test control solution of 0.5ml (0.45ml bovine serum albumin and distilled water 0.05ml), the product control solution of 0.5ml (0.45ml of distilled water and 0.05ml of test sample solution) and the standard solution of 0.5ml (0.45ml of Bovine serum albumin and 0.05ml of Diclofenac sodium). All these solutions were maintained at pH 6.3 using 1N HCl and incubated at 37°C for 20 min and then continued at increased temperature of 57°C for 3min. After cooling the solutions, 2.5ml of phosphate buffer was added, then the absorbance was measured by UV Visible spectrophotometer at 416nm (Perkin Elmer, USA). The percentage inhibition of protein denaturation was calculated using the formula:

Percentage inhibition =

$$\frac{[100 - (\text{optical density of test solution} - \text{optical density of product control})]}{(\text{optical density of test control})} \times 100$$

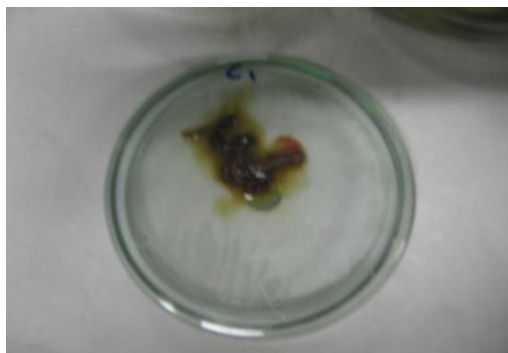
Table 1: Anthelmintic activity and anti-arthritis activity of ethanolic extract of *Nymphoides macrospermum vasud*

S. No.	Materials	Quantity of Extract (µL)	Time taken for paralysis (min)	Time taken for death (min)
1.	Ethanolic extract of <i>Nymphoides macrospermum vasud</i>	250	2	4
		500	1	2
		1000	-	1
2.	Metronidazole	10(mg/ml)	5	13.2
3.	Control	-	-	-

Table 2: In-vitro Anti -arthritic activity of ethanolic extract of *Nymphoides macrospermum vasud*

S.No	Drug concentration in $\mu\text{g/ml}$	In-vitro Anti -arthritic activity (%)	
		Diclofenac sodium	Ethanolic extract of <i>Nymphoides macrospermum</i>
1.	100	94.22	20
2	300	99.156	31.43

A)



B)



C)



D)

**Figure 1: Anthelmintic activity of ethanolic extract of *Nymphoides macrospermum vasud*, A-250 μL , B-500 μL , C1000 μL -, D-Control (water).****RESULT AND DISCUSSION:**

The phytochemical investigations of *Nymphoides macrospermum vasud* reveals the presence of tannins (Vedha Hari *et.al* 2010) and other phytoconstituents, which are the major components causing the paralysis and death of larva by directly inhibiting the metabolism. The anthelmintic activity of extract was carried by well established procedure and the data was tabulated (Table 1). It shows that the time taken for paralysis was 2min, 1min, <1 min and time taken for death was 4min, 2min, and 1min for the concentrations of 250 μL 500 μL and 1000 μL of extracts respectively. It is compared to the standard drug Metronidazole (10mg/ml) which had taken 5min for paralysis and 13.2min for causing death. Based on the above data the

activity of ethanolic crude extract of *Nymphoides macrospermum vasud* was reported to be better compared to the standard drug commercially available in the market (figure 1). The same extract of *Nymphoides macrospermum vasud* showed relatively less effect against arthritis (maximum of 31% inhibition with 300 μg of extract) compared to standard drug Diclofenac showing 99% inhibition. From the above results, it is concluded that the ethanolic extract of *Nymphoides macrospermumvasud* can be used as for the effective treatment of helminthiasis. Also the arthritis activity is reported to be less effective (Table 2).

REFERENCES:

1. Vasudevan R. A new species of Nymphoides (Menyanthaceae) from south India. Kew Bull 1968; 22: 101-106.
2. Anita M., Sudha C., Madhavan V., Yoganarasimhan S.N. Anticonvulsant and Sedative Activity of Tagara (Nymphoides macrospermum). J Pharma Bio 2006; 45: 407-410.
3. Neelakanthan V.T., Yasmin B.A., Nagappan RM, Vedha Hari BN, Sudha C, investigation of antimicrobial activity of *nymphoides macrospermum* *Indian Journal of Natural Sciences* 2010; 1(1): 53-56.
4. Vedha Hari B.N. Saravana Kumar. P., Ramya Devi D. *In-Vitro* Anthelmintic Activity of the Latex of *Ficus Religinosa*, *Ficus Elastica* And *Ficus Bengalensis* J Phytol; 2011;3(3): 26-30.
5. Gopinath N, Harish G. Ashwin G, Kumar, Chitra K., Reddy U.M.C, Investigation of In-vitro Anti-arthritis, anti-inflammatory and Anti-Oxidant activity of Tectona Grandis Linn Indian Drugs. 2010; 47(6): 74-76.
