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Assessment of macrofilaricidal activity of leaf extracts of *Terminalia* sp. against bovine filarial parasite *Setaria cervi*

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ABSTRACT

Antifilarial potential of three medicinal plants namely, *Terminalia bellerica*, *Terminalia chebula* and *Terminalia catappa* was explored using *Setaria cervi*, a bovine filarial parasite at concentrations of 2.5, 5 and 10 mg/ml. Amongst all the extracts, methanol extract of *T. bellerica* showed highest macrofilaricidal activity i.e. 84.63 ± 1.11 at 10 mg/ml in MTT reduction assay with IC_{50} value of 2.7 mg/ml. which was better than the standard DEC i.e. 79.22 ± 3.1% at 10 mg/ml with IC_{50} value 2.84 mg/ml. Other plant extracts showed mild *in vitro* macrofilaricidal activity. *T. bellerica* methanol extract exhibited significant GST activity of 18.86 ± 0.21 and 12.83 ± 0.03 μM/ml/min at 5 and 10 mg/ml with percentage inhibition value of 73.96% and 82.29% respectively. DEC showed GST activity value of 40.03 ± 4.14 and 21.48 ± 6.44 μM/ml/min with percentage inhibition value of 21.76% and 58.01% at 5 and 10 mg/ml respectively. Thus, methanol extract of leaves of *T. bellerica* exhibited highly significant antifilarial potential and needs detailed analysis.

Introduction

Filariasis is a major public health problem affecting over 120 million people in Central Africa, Central and South Africa and large regions of Asia including China and India [1]. Currently, 1.10 million people in 55 countries are living in areas that require preventive chemotherapy to stop the spread of infection. The current drug regime for lymphatic filariasis include diethylcarbamazine (DEC), albendazole and ivermectin. These drugs have been consistently used in the MDA (Mass Drug Administration) programme to block the disease transmission in the endemic areas. These drugs are active against blood infesting microfilariae (mf) thus efficient in interrupting transmission of the disease; however, they are less effective against the adult worm [2,3]. These drugs have some side effects as well, such as nausea, vomiting, gastric disturbances and giddiness [4]. Owing to this still there is a need for safe, non toxic and macrofilaricidal drug. Secondary metabolites from plants have always served as potential source of alternative medicines. In this study, three plants of Terminalia genus namely, *Terminalia bellerica*, *Terminalia chebula* and *Terminalia catappa* were explored for their antifilarial activities using *Setaria cervi*, a bovine filarial parasite as target. *Terminalia* sp., belongs to family Combretaceae, consisting of approximately 150 species of trees and shrubs [5]. The leaves and bark are well known for their medicinal potential [6] whereas fruits of *T. bellerica* and *T. chebula* are used in Ayurvedic medicine "Triphala", which is a powerful antioxidant and detoxifier [7]. *T. catappa* leaves contain several bioactive compounds responsible for antiviral, antibacterial, antifungal and anticancerous activities [8]. Keeping in view of medicinal potential of all the three species same were explored for their antifilarial potential for the development of a safe and non toxic alternative for present day synthetic drugs.

Materials and methods

Collection and processing of plant material

The leaves of the medicinal trees namely *T. bellerica*, *T. chebula* and *T. catappa* were collected from Regional Plant Resource Centre, Bhubaneswar, Odisha, India. Solvent extracts were prepared using the standard protocols [9].

Collection of Setaria worms

The bovine *S. cervi* adult parasites were collected from the peritoneal cavity of the freshly slaughtered cattles in the slaughter house of Nandankanan Zoological Park, Bhubaneswar, Odisha, India.

Worm motility inhibition assay

In vitro worm motility inhibition assay was performed by the Standard method [10]. The worms were transferred to RPMI-

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1640 medium and supplemented with 5% (v/v) heat-inactivated Fetal bovine serum. Stock solution of strength 200 mg/ml was prepared of solvent leaf extracts and standard DEC in DMSO (Dimethyl sulphoxide). Two adult worms were introduced into each vial and were subjected to solvent extracts at concentrations of 2.5–10 mg/ml. A simultaneous positive control was kept without the test solution but with equal volume of DMSO as in test vials. Experimental and controls were incubated at 37 °C for 24 h. The motility readings were taken in each hour interval up to four hours to examine the drug activity on worms. After 24 h exposure, the worms were transferred to fresh PBS (1×) to check the motility of the worms. If the worms did not regain their motility, then activity was considered irreversible and showed that the drugs used were active against filarial worms. Motility assay was followed by MTT reduction assay.

MTT reduction assay

Effect of solvent extracts on adult female Setaria worm was studied by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] formazan reduction assay [11]. After worm motility inhibition study, the treated worms were washed in fresh PBS (1×) pH 7.4. Then the parasites were transferred to 24 well culture plates containing 0.01% MTT prepared in PBS (1×) and incubated at 37 °C for 1h duration. After incubation the treated worms were transferred to chilled PBS (1×). PBS was removed by drying the worm on blotting paper. Worms were carefully transferred to 24 well culture plates containing 2 ml DMSO and incubated at 37 °C for 2 h. The absorbance of the resulting formazan solution was then determined at 510 nm in a microplate reader. Percent inhibition was calculated as follows:

$$\text{Percentage inhibition} = \text{Control} - \text{Sample}/\text{Control} \times 100$$

IC_{50} value of standard drug and extracts were compared for effective extracts.

Glutathione S-transferase (GST) inhibition assay

GST inhibition study was carried out by the standard method [12]. For GST assay, worms were washed thoroughly with phosphate-buffered saline (PBS), pH 7.4, prepared a 10% homogenate, centrifuged at 10,000 × *g* for 30 min at 4 °C, supernatant containing the cytosolic fraction was used for GST assay. GST activity was measured according to the method of Habig [12] using 1-chloro-2,4-ditnitrobenzene (CDNB) and GSH as substrates. The protein content in the crude homogenate of the worm extract was determined [13] using bovine serum albumin as standard protein. The cytosolic fraction was incubated with concentrations 5 and 10 mg/ml of each extract/standard drug for 10 min at room temperature in the reaction mixture containing 465 μl Assay buffer

Table 1
In vitro macrofilaricidal activity of plant extracts/DEC against adult *Setaria cervi* in terms of motility inhibition.

Plants used	Extracts	Dose (mg/ml)	Motility score of adult worms (in h)				
			1 h	2 h	3 h	4 h	24 h
Terminalia bellerica	Hexane	**2.5**	4+	4+	4+	4+	4+
		5	4+	4+	4+	4+	4+
		10	4+	4+	4+	4+	4+
	Chloroform	**2.5**	4+	4+	4+	4+	4+
		5	4+	4+	4+	4+	3+
		10	4+	4+	4+	3+	2+
	Acetone	**2.5**	4+	4+	4+	3+	3+
		5	4+	4+	4+	3+	2+
		10	4+	4+	4+	3+	2+
	Methanol	**2.5**	4+	4+	4+	4+	1+
		5	4+	4+	3+	2+	1+
		10	4+	4+	3+	1+	1+
Terminalia catappa	Hexane	**2.5**	4+	4+	3+	2+	1+
		5	4+	4+	1+	1+	1+
		10	4+	1+	1+	1+	1+
	Chloroform	**2.5**	4+	3+	2+	1+	1+
		5	4+	1+	1+	1+	1+
		10	4+	1+	1+	1+	1+
	Acetone	**2.5**	4+	4+	4+	4+	1+
		5	4+	4+	4+	4+	1+
		10	4+	4+	4+	4+	1+
	Methanol	**2.5**	4+	4+	4+	4+	1+
		5	4+	4+	4+	4+	1+
		10	4+	4+	2+	1+	1+
Terminalia chebula	Hexane	**2.5**	4+	4+	3+	2+	2+
		5	4+	3+	1+	1+	1+
		10	4+	1+	1+	1+	1+
	Chloroform	**2.5**	4+	1+	1+	1+	1+
		5	4+	1+	1+	1+	1+
		10	4+	1+	1+	1+	1+
	Acetone	**2.5**	4+	4+	4+	2+	1+
		5	4+	4+	3+	2+	1+
		10	4+	3+	1+	1+	1+
	Methanol	**2.5**	4+	4+	4+	4+	1+
		5	4+	4+	4+	3+	1+
		10	4+	4+	3+	3+	1+
DEC (standard)		**2.5**	4+	4+	4+	4+	1+
		5	4+	4+	4+	4+	1+
		10	4+	1+	1+	1+	1+

Motility readings: 4+ (highly motile), 3+ (motile), 2+ (sluggish), 1+ (non-motile).

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