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Total accepted phenolic, tannin, triterpenoid, flavonoid and sterol contents, anti-diabetic, anti-inflammatory and cytotoxic activities of *Tectaria paradoxa* (Fee.) Sledge

Manivannan V, Johnson M *

Centre for Plant Biotechnology, Department of Botany, St. Xavier's College (Autonomous), (Affiliated to Manonmanaim Sundaranar University, Tirunelveli- 627 012, Tamil Nadu, India), Palayamkottai 627 002, Tamil Nadu, India

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ABSTRACT

The present study was aimed to reveal the phytochemical composition and bio potentials of *Tectaria paradoxa* (Fee.) Sledge. The total phenolic, tannin, flavonoid, terpenoids, sterols content were determined. RBC membrane stabilization against heat induced haemolysis, *In-vitro* Alpha-amylase inhibitory assay and Brine Shrimp lethality bioassay was performed to determine the anti-inflammatory, anti-diabetic and cytotoxic activity. Among the tested extracts, methanolic extracts of *T. paradoxa* showed high amount of phenolics 351.43 ± 14.5 mg GAE/g, tannin 34.38 ± 1.02 mg GAE/g, flavonoids 1384.44 ± 50.92 mg QE/g, triterpenoids 130.5 ± 2.77 mg/g and acetone extracts of *T. paradoxa* displayed maximum amount of sterols 3.2 ± 0.2 mg/g. The extracts of *T. paradoxa* demonstrated dose dependent anti-inflammatory, anti-diabetic and cytotoxic activities. The anti-inflammatory activity of the *T. paradoxa* were as follows methanol > chloroform > acetone > petroleum ether. The anti-diabetic properties of the *T. paradoxa* were as follows methanol > acetone > chloroform > petroleum ether. The cytotoxicity of the *T. paradoxa* were as follows chloroform (LC_{50} = 25.52 µg/mL) > petroleum ether (LC_{50} = 36.99 µg/mL) > methanol (LC_{50} = 44.26 µg/mL) > acetone (LC_{50} = 55.9 µg/mL). The existence of phenolics, tannin, flavonoids, sterols and triterpenoids may be responsible for the observed biological activities. The results of the present study identified the pool of medicinal properties existence in *T. paradoxa*. Further studies on the isolation of active principles may bring out an alternative source for anti-inflammatory and anti-cancer drugs from *T. paradoxa*.

1. Introduction

Since the time immemorial the medicinal value of pteridophytes is known to man. The rhizome of *Tectaria cicutaria* has been used in Ayurveda for the treatment of various disorders [1,2]. The decoction of *Tectaria cicutaria* is employed for the treatment of various types of gynecological disorders as well as inflammatory conditions. Many researchers have confirmed the pharmacological activities of pteridophytes [3–11]. The metabolites phenolics, flavonoids, alkaloids and terpenoids are responsible for the biopotency of the ferns [12–15]. Preeti and Namdeo [10] subjected *Tectaria cicutaria* rhizomes, to phytochemical analysis, anti-microbial activity and *in-vitro* anticancer activity and confirmed the presence of bioactive metabolites in the extracts. They observed the antimicrobial activity against *Proteus vulgaris*. The ethanolic extract of *Tectaria cicutaria* rhizomes showed excellent anticancer activity against Human Leukemia Cell Line (K562) with GI_{50} value 11.9 µg/mL. Castrejón-Arroyo et al., [8] evaluated the anti-inflammatory activity and antioxidant capacity, total phenolic and flavonoid contents of *T. heracleifolia* raw extracts. The *T. heracleifolia* raw extracts showed the anti-inflammatory activity with 52 % and 0.084 mg/ml was required to obtain a 50 % antioxidant effect (IC_{50}). Pawar et al., [16] revealed the phytochemical profiles of *Tectaria coadunata*. Preeti and Namdeo [17] studied the anticancer action of *Tectaria cicutaria* in human cancer cell lines. Johnson et al., [18] have observed the inter-specific variation among the three *Tectaria* species using isoperoxidase analysis. But there is no report on the phytochemical composition and biological activities of *Tectaria paradoxa* (Fee.) Sledge. With this knowledge the present study was aimed to reveal the phytochemical composition and bio potentials of *Tectaria paradoxa* (Fee.) Sledge.

* Corresponding author.
E-mail address: ptcjohnson@gmail.com (J. M).

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2. Materials and methods

2.1. Collection of materials

Healthy, disease free plant samples of *Tectaria paradoxa* (Fee.) Sledge were collected from high altitude semi-evergreen forest ranges of Tirunelveli district, Tamil Nadu, India.

The *Tectaria paradoxa* was identified using the standard flora and authenticated by Dr. M. Johnson. *Tectaria paradoxa* (Fee.) Sledge voucher specimen was deposited in Centre for Plant Biotechnology Herbarium, St. Xavier's College (Autonomous), Palayamkottai, India. To remove the soil particles and other debris, the collected plants *T. paradoxa* were brought to the laboratory and washed well with running tap water for 10 min. The washed *T. paradoxa* were blotted on the blotting paper and spread out at room temperature under shade for a period of fifteen days. The shade dried *T. paradoxa* were ground to fine powder using tissue blender. The powdered *T. paradoxa* were then stored in refrigerator at 4 °C for further use.

2.2. Preparation of extracts

30 g of dried and powdered whole plant materials of *T. paradoxa* were extracted with 180 ml of petroleum ether (45 °C), chloroform (55 °C), acetone (52 °C) and methanol (75 °C) by using Soxhlet extractor for 8 h at a temperature not exceeding the boiling point of the solvent. All extracts were frozen and freeze dried. The powder was stored in an amber bottle and stored at 4 °C in a refrigerator for later biological activities. For quantitative analysis and biological activities, the extracts were disolved in DMSO (w/v) (5 mg of crude petroleum ether, chloroform, acetone and methanolic extracts of *T. paradoxa* were disolved in 5 ml of DMSO (mg/mL)).

2.3. Phytochemical analysis

The crude extracts were screened for the occurrence or absence metabolites by the standard method described by Harborne [19]. The total phenolic, tannin, flavonoid, terpenoids, sterols content were determined according to the method described by Siddhuraju and Becker [20], Zhishen et al., [21], Feng et al. [22], respectively.

2.4. Biological activities

RBC membrane stabilization against heat induced haemolysis was performed to determine the anti-inflammatory activity of *T. paradoxa* extracts [23,24]. *In-vitro* alpha-amylase inhibitory assay was carried out to determine the anti-diabetic properties of *T. paradoxa* extracts [25]. Brine Shrimp lethality bioassay was performed to examine the cytotoxic properties of *T. paradoxa* extracts [26]. The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental studies [27].

To validate the observed results the statistical analysis was performed using SPSS 21 software. Pearson correlation test was performed between the metabolites concentration and biological activities. The correlation is significant at the 0.01 level (2-tailed). To determine the significance, the t - test was performed between the metabolites concentration and biological activities.

3. Results

Among the tested extracts, methanolic extracts of *T. paradoxa* showed high amount of phenolics 351.43 ± 14.5 mg GAE/g, tannin 34.38 ± 1.02 mg GAE/g, flavonoids 1384.44 ± 50.92 mg QE/g, triterpenoids 130.5 ± 2.77 mg/g and acetone extracts of *T. paradoxa* displayed maximum amount of sterols 3.2 ± 0.2 mg/g (Table 1). The total phenolics, tannin, flavonoids and triterpenoids contents of *T. paradoxa* extracts were as follows methanol > chloroform > acetone > petroleum ether. The extractable sterols of *T. paradoxa* were as follows acetone > methanol > petroleum ether > chloroform (Table 1).

Table 1
Secondary Metabolites of *Tectaria paradoxa*.

Metabolites	Methanol	Chloroform	Pet. Ether	Acetone
Phenolics (mg GAE / g)	351.43 ± 14.5	334.76 ± 7.95	288.89 ± 8.21	332.86 ± 7.14
Flavonoids (mg QE / g)	1384.44 ± 50.92	1104.44 ± 62.92	706.67 ± 10.72	1095.56 ± 59.66
Sterols (mg / g)	2.94 ± 0.07	2.27 ± 0.11	2.83 ± 0.19	3.2 ± 0.2
Tannin (mg GAE / g)	34.38 ± 1.02	25.48 ± 1.37	5.3 ± 0.61	9.83 ± 0.65
Triterpenoids (mg / g)	130.5 ± 2.77	122.5 ± 2.25	102.33 ± 2.42	105.5 ± 4.17

The biopotency of *T. paradoxa* extracts were determined by alpha glucosidase, heat induced haemolysis and brine shrimp biolethality bioassay. The extracts of *T. paradoxa* demonstrated dose dependent toxicity (brine shrimp lethality bioassay), anti-inflammatory and anti-diabetic activities (Fig. 1–3). The anti-inflammatory activity of the *T. paradoxa* were as follows methanol ($t = 0.02$) > chloroform ($t = 0.001$) > acetone ($t = 0.001$) > petroleum ether ($t = 0.001$) (Fig. 1). 100 µg/mL of standard indomethacin was dispalyed 71.43 % inhibition. A strong correlation ($r = 0.998$) between chloroform and petroleum ether extracts of *T. paradoxa* and anti-inflammatory activities was attained. Next to that $r = 0.996$ correlation coeeficient was obtained between acetone and anti-inflammatory activities. Correlation coeeficient of $r = 0.967$ was obtained between methanolic extracts of *T. paradoxa* and anti-inflammatory activities. The correlation is significant at the 0.01 level (2-tailed). The anti-diabetic properties of the *T. paradoxa* were as follows methanol ($t = 0.000$) > acetone ($t = 0.003$) > chloroform ($t = 0.000$) > petroleum ether ($t = 0.004$) (Fig. 2). 78 % of activity was observed in the standard acarbose at 500 µg/mL. A strong positive correlation ($r = 0.973$) was obtained between methanolic extracts of *T. paradoxa* and anti-diabetic activities, $r = 0.987$ for chloroform, $r = 0.963$ for acetone and $r = 0.958$ for petroleum ether. The cytotoxicity of the *T. paradoxa* were as follows chloroform ($LC_{50} = 25.52$ µg/mL; $t = 0.003$) > petroleum ether ($LC_{50} = 36.99$ µg/mL; $t = 0.009$) > methanol ($LC_{50} = 44.26$ µg/mL; $t = 0.008$) > acetone ($LC_{50} = 55.9$ µg/mL; $t = 0.003$) (Fig. 3). The standard plumbagin showed 100 % mortality of brine shrimp nauplii at 0.046 mg/mL. A strong positive correlation ($r = 0.985$) was observed between concentrations of methanolic extracts and cytotoxicity of *T. paradoxa*, $r = 0.946$ for chlorofom, $r = 0.986$ for acetone and $r = 0.993$ for petroleum ether. The studied extracts of *T. paradoxa* showed significant lethality against brine shrimp (Table 2; Fig. 3).

4. Discussion

Phenolic compounds and tannins are known to possess anti-inflammatory, anti-oxidant, anti-microbial, insecticidal, anti-diabetic [28], wound healing, anti-diuretic, anti-parasitic, cytotoxic and anti-neoplastic activities [29]. Flavonoids show anti-allergic, anti-inflammatory, anti-microbial and anticancer activity [30,31]. Steroids and saponins are the sub- groups of triterpenoids. Saponins possess antimicrobial and anti- inflammatory activity [32]. The results of the present study also confirm the presence of phenolics, tannin, flavonoids, sterols and triterpenoids with varied amount in the studied extracts of *T. paradoxa*. The existence of these metabolites may be responsible for the observed biological activities. *T. paradoxa* extracts showed anti-diabetic, anti-inflammatory and cytotoxic activities with varied frequencies. The varied frequency activities may be due to the variation in metabolites contents. The concentrations and frequency of activities are directly correlated. Brine Shrimp Lethality Bioassay (BSLB) has been successfully employed as a simple biological tool to identify the

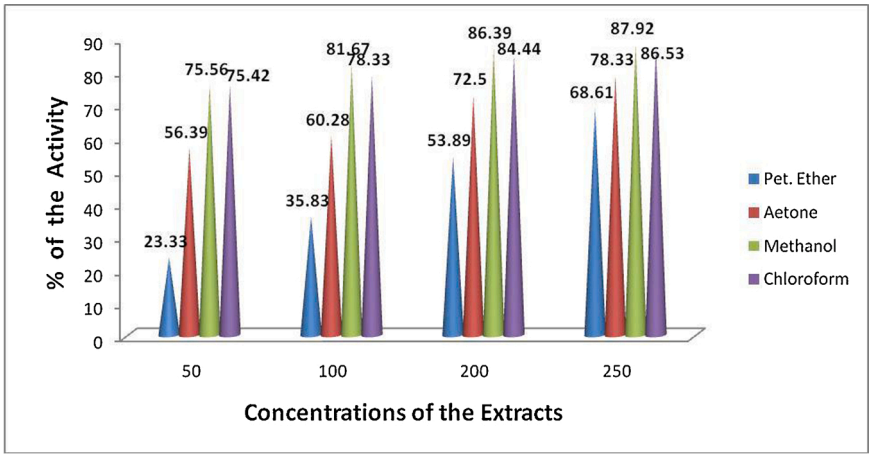

Fig. 1. Anti-inflammatory Activity of *T. paradoxa.*

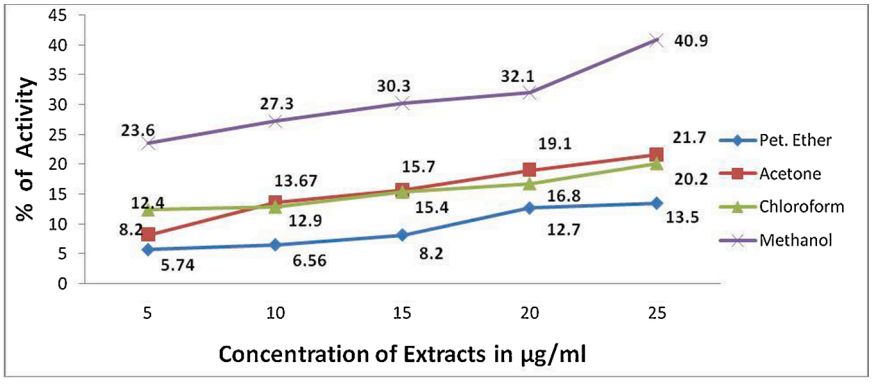

Fig. 2. Anti-Diabetic Activity of *T. paradoxa.*

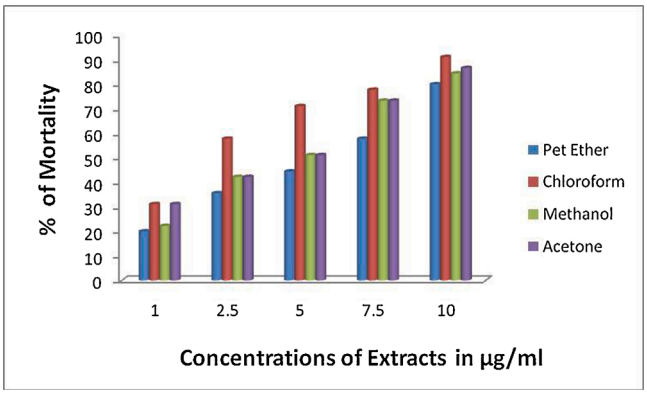

Fig. 3. Cytotoxicity of *T. paradoxa.*

antitumour compounds / fractions / crude extracts of plants [33]. The BSL bioassay has good correlation with the human solid tumour cell lines [34]. The studied extracts of *T. paradoxa* can be considered as a promising candidate for a plant-derived anti-tumour compound. LC_{50} values < 1000 ppm are considered significant for crude extracts [35]. The cytotoxicity of the *T. paradoxa* were displayed less than LC_{50} values < 1000 ppm viz., chloroform (LC_{50} = 25.52 µg/mL) > petroleum ether (LC_{50} = 36.99 µg/mL) > methanol (LC_{50} = 44.26 µg/mL) > acetone (LC_{50} = 55.9 µg/mL). The crude extracts of plants with LC50 values <1000 µg/ml using BSLB are recognized to hold various physiologically active principles [36]. The existence of phytoconstitutents viz., alkaloids, phenolics and terpenoids in plant extracts has been associated with anticancer and cytotoxic activity [35–37]. The results of the present study suggested that *T. paradaxa* extracts treatment against *Artemia salina* induced a dose dependent lethal effect. The *T. paradaxa* extracts treated *Artemia salina* showed the morphological changes, which disrupted and affected the swimming ability, feeding, intestinal enlargement, deformation and loss of antennae in *A. salina.* The *A. salina* cultured in the control failed to show the morphological change. The exposure of *T. paradaxa* extracts may generate the reactive oxygen species (ROS) that may cause cytotoxicity. Similar kind of observations was observed in the aqueous and silver nanoparticles of *O. chinensis* [38]. Johnson et al. [36,38] and Nirmali et al. [39] employed *in-vitro* alpha amylase inhibitory activity to predict the antidiabetic potential of *Sphaerostephanos unitus*, *Odontosoria chinensis* and *Adenanthera pavonina* extracts respectively. In the present study also *in-vitro* alpha amylase inhibitory activity was adopted and identified the antidiabetic potetnials of *T. paradoxa.* The anti-inflammatory activity of *Sphaerostephanos unitus*, *Odontosoria chinensis* and *Gardenia coronaria* leaves extracts was assessed by *in vitro* HRBC membrane stabilization method [36,38,40]. Johnson et al. [36,38] employed the *in-vitro* alpha-amylase inhibitory assay and Brine Shrimp lethality bioassay to determine the toxicity and anti-diabetic properties of *Sphaerostephanos unitus* and *Odontosoria chinensis*. In the present study also *in vitro* HRBC membrane stabilization against heat induced haemolysis method and *in-vitro* alpha-amylase inhibitory assay and Brine Shrimp lethality bioassay are employed and determined the toxicity, anti-diabetic and anti-inflammatory properties of *T. paradoxa.* The results of the present study identified the pool of medicinal properties existence in *T. paradoxa*. Further studies on the isolation of active principles may bring out an alternative source for anti-inflammatory and anti-cancer drugs from *Tectaria paradoxa*.

CRediT authorship contribution statement

Manivannan V: Data curation, Investigation, Methodology. **Johnson M:** Data curation, Methodology, Supervision, Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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