COMPARATIVE PHYTOCHEMICAL AND ANTIBACTERIAL EFFICACY OF *TECTARIA COADUNATA* AND *TECTARIA WIGHTII*

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Abstract: Ferns are considered as the least explored and neglected plant group, even though they are the second largest community of vascular plants. Compared to flowering plants, they had not yet received much attention on the aspects of phytochemistry and antimicrobial studies. The present study aims at evaluating the phytochemical and antibacterial efficacy of different solvent extracts of *Tectaria coadunata* C. Chr and *Tectaria wightii* Ching. Plant parts like fronds and rhizome are extracted by hot solvent extraction method with soxhlet apparatus using petroleum ether, ethyl acetate, methanol and water. Preliminary phytochemical screening revealed the presence of various secondary metabolites such as alkaloids, cardioglycosides, phenolics, saponins, tannins, terpenoids, steroids, quinones, flavonoids and glycosides. Extracts are subjected to antimicrobial assay against six pathogenic bacterial strains such as *Klebsiella pneumoniae, Salmonella typhimurium, Pseudomonas aeruginosa, Escherichia coli, Listeria monocytogenes* and *Xanthomonas campestris*. Ethyl acetate and methanol extracts of fronds and rhizome showed significant antibacterial activity against the strains tested. Extracts of fronds showed higher effects rather than the extracts of rhizome. While comparing both species, *T. wightii* has more potential antibacterial effect than *T. coadunata*. The present study is an attempt to make some advancement in this direction and will give impulsion for further research on phytochemical evaluation and bioactive compound isolation of the fern species.

Key words: Pteridophytes, Phytochemical, Antibacterial, *Tectaria*

I. INTRODUCTION

Pteridophytes, the seedless vascular plants, had a very flourishing past in dominating the vegetation on the earth about 400 million years ago. Although they are largely replaced by the seed bearing vascular plants in the extant flora today, yet they constitute a prominent part of the present day vegetation in the world particularly in the tropical countries. They represent an important part of the Indian flora. The pteridophytic population of political India is about 10% of the total pteridophytic flora of the world (Fraser- Jenkins, 2008). Pteridophytes are resistant to microbial infections which may be one of the crucial factors for their evolutionary success and fact that they lasted for more than 350 million years (Sharma and Vyas, 1985).

Recent studies proved that fronds and rhizome of pteridophytes are the rich source of various secondary secondary metabolites. The phytochemicals such as alkaloids, flavonoids, phenols, phytosterols, gums, tannins and saponins present in the methanolic extracts of *Christella dentata* are known to possess medicinal and therapeutic properties. The fern has also antimicrobial potential against *Rhodococcus pyridinivorans* and *Geobacillus stearothermophilus* (Manhas *et al*., 2018).

The phytochemical, antibacterial and in-vitro anticancer activity studies of *Tectaria cicutaria* was carried out by Preeti and Namdeo (2018) revealed the presence of significant amount of phenolic and flavonoid contents of the plant. The plant possesses antibacterial activity against *Proteus vulgaris* and excellent anticancer activity against leukemia cell line. *Asplenium nidus*, a threatened ethno-medicinal fern of North East India, shows strong inhibitory effect against pathogenic bacterial strains such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Nath et al., 2013).

Tectaria coadunata C. Chr and *Tectaria wightii* Ching belongs to the Class Polypodiopsida, Order Polypodiales and Family Tectariaceae. The present work is aimed at comparing and evaluating the phytochemical analysis and antibacterial properties of the rhizome and frond extracts of *T. coadunata* and *T. wightii*.

II. MATERIALS AND METHODS

2.1 Collection of plant material

Specimens for the present study were collected from Cheruthony and Munnar, Idukki District, Kerala. The plants were authenticated and specimens were deposited in the herbarium of Kerala Forest Research Institute, Thrissur (No. 18092 and 18093).

2.2 Preparation of extracts

The specimens were washed thoroughly, and the rhizome and frond were separated, shade dried and powdered with a blender. Extracts were prepared using soxhlet extractor. 30 g of powdered samples were extracted successively with 150 ml of petroleum ether, ethyl acetate, methanol and distilled water for 8–12 h at a temperature not exceeding the boiling point. The extracts were concentrated in vacuum using a rotary evaporator.

2.3 Qualitative phytochemical screening

Qualitative phytochemical screening was performed on the extracts to detect the presence of phytoconstituents (Harborne, 1998).

2.3.1 Test for Alkaloids

1 ml of plant extract was mixed with 1 ml of 1 % HCl, warmed and filtered. 2 ml of filtrate was treated with Mayer's reagent. Turbidity or precipitation, green color indicates the presence of alkaloid.

2.3.2 Test for Cardio glycoside

To 1 ml of extract, 2 ml of glacial acetic acid was added and few drops of 5% ferric chloride were also added. This was under layered with 1 ml concentrated sulphuric acid. Formation of brown ring at the interface or yellow color indicates the presence of cardio glycosides.

2.3.3 Test for Phenolic compounds

To a few drops of 5% glacial acetic acid, 1 ml of plant extract and few drops of 5% sodium nitrite were added. Formation of muddy brown color indicates the presence of phenols.

2.3.4 Test for Saponin

0.5 g of plant extract was dissolved in 2 ml of boiling water in a boiling tube, allowed to cool and shaken well to mix. The appearance of foam indicates the presence of saponin.

2.3.5 Test for Tannin

To 2 ml of the test solution, 2 ml of ferric chloride was added. Presence of blue black or dark green colour indicates presence of tannin.

2.3.6 Test for Terpenoid

To 1 ml of extract, 2 ml of chloroform and 1.5 ml of concentrated sulphuric acid were added carefully. Formation of reddish brown color indicates the presence of terpenoid.

2.3.7 Test for Steroids

To 1 ml plant extract, 2 ml of chloroform and 1 ml of sulphuric acid were added. Formation of reddish brown color at the interface indicates the presence of steroids.

2.3.8 Test for Quinone

To 1 ml of extract, 1 ml of concentrated sulphuric acid was added. Formation of red color indicates the presence of quinone.

2.3.9 Test for Flavonoid

To 3 ml of extract, 4 ml of 1N NaOH was added. Formation of dark yellow color indicates the presence of flavonoid.

2.3.10 Test for Glycoside

To 1 ml of glacial acetic acid, 1 ml plant extract was added and cooled. Two drops of ferric chloride were added, followed by careful addition of concentrated sulphuric acid along the walls of test tube. Formation of reddish brown color ring at the junction of two layers indicates the presence of glycoside.

2.3.11. Test for Anthocyanin/ Betacyanin

To 2 ml extract, 1 ml of 2 N NaOH was added. Allowed to heat for 5 minutes at 100^0C. Formation of bluish green color indicates the presence of anthocyanin, whereas yellow color indicates the presence of betacyanin.

2.4 Test for antibacterial activity

The agar disc diffusion method was used to evaluate the antibacterial activity (Murray *et al*., 1957). The antibacterial activity of different extracts was tested on pathogenic bacterial strains like *Klebsiella pneumoniae* (NCIM 2883), *Salmonella typhimurium* (NCIM 2501), *Pseudomonas aeruginosa* (NCIM 5210), *Escherichia coli* (NCIM 5846), *Listeria monocytogenes* (NCIM 5260) and *Xanthomonas campestris* (NCIM 5028) obtained from CSIR National Chemical Laboratory, Pune. Standard antibiotic discs such as amoxyclav (30 mcg) and methicillin (5 mcg) were used as positive control. Different solvents like petroleum ether, ethyl acetate, methanol and distilled water were used as negative control. The test for antibacterial activity was carried out by measuring the diameter of inhibition zone (millimetre). The experiment was repeated for three times and the results were the mean of three replicates.

III. RESULTS AND DISCUSSION

3.1 Qualitative phytochemical screening

Table 1 shows the findings of preliminary phytochemical screening of *T. coadunata* whereas Table 2 shows the results of *T. wightii*. Extracts of rhizome and fronds showed the existence of various phytoconstituents such as alkaloids, cardioglycosides, phenols, saponins, tannins, terpenoids, steroids, quinones, flavonoids, glycosides anthocyanin and betacyanin. Ethyl acetate, methanol and aqueous extracts yielded more secondary metabolites than petroleum ether extracts.

Table 1: Preliminary phytochemical analysis of *T. coadunata*

Phytoconstituents	Rhizome				Fronds			
	PE	EA	M	A	PE	EA	M	A
Alkaloids	++	+++	-	+++	++	+++	-	+++
Cardio glycoside	+++	+++	++	+	+++	-	++	+
Phenol	-	-	+++	++	-	++	+++	++
Saponin	++	++	+++	+	++	+	+++	+
Tannin	-	+	+++	+++	+	++	+++	++
Terpenoid	-	+++	+++	+++	-	++	+++	+++
Steroid	-	+++	+++	++	-	+	+++	++
Quinone	+	++	+++	++	-	-	+++	++
Flavonoid	+	+	+++	++	+	+++	+++	+
Glycoside	++	++	+++	+	+++	-	+++	++
Anthocyanin	-	-	-	-	-	++	-	-
Betacyanin	+	-	-	-	++	+	+	++

Table 2: Preliminary phytochemical analysis of *T. wightii*

Phytoconstituents	Rhizome				Fronds			
	PE	EA	M	A	PE	EA	M	A
Alkaloids	++	+++	+	-	+++	+++	-	+++
Cardio glycoside	++	++	+++	++	++	++	+++	+
Phenol	-	-	+++	++	-	++	+++	++
Saponin	+	+	+++	++	+++	+	+++	++
Tannin	+	+	+++	+++	++	++	+++	+++
Terpenoid	+	++	+++	+++	+	+	+++	+++
Steroid	-	++	+++	+++	-	+	+++	++
Quinone	-	+	+++	++	-	+	+++	+++
Flavonoid	+	+	+++	++	+	++	+++	++
Glycoside	+++	++	+++	++	+++	+	+++	++
Anthocyanin	-	-	-	-	-	+	-	-
Betacyanin	-	+	-	-	++	++	-	-

(PE- Petroleum ether, EA- Ethyl Acetate, M- Methanol and A-Water)

(+++: Strongly positive; ++: Moderately positive; +: Positive; -: Negative)

3.2 Evaluation of antibacterial assay

Table 3: Antibacterial activity of *T. coadunata*

Bacterial Strains	Zone of inhibition (mm): Values = Mean ± Standard Deviation									
	Rhizome				Fronds				Antibiotic	
	PE	EA	M	A	PE	EA	M	A	Methicillin (5 mcg)	Amoxyclav (30 mcg)
K. pneumoniae	0	8 ± 0	0	0	0	7 ± 0	0	0	0	0
S. typhimurium	0	0	9.33 ± 0.57	0	0	0	10.16 ± 0.28	0	0	8 ± 0.1
P. aeruginosa	0	0	7.33 ± 0.28	0	0	0	8 ± 0	0	0	7 ± 1
E. coli	0	0	0	0	0	7.33 ± 0	9.66 ± 0.57	0	0	7.66 ± 0.57
L. monocytogenes	0	0	7 ± 0	0	0	0	8.33 ± 0.28	0	0	6.83 ± 0.28
X. campestris	0	0	9 ± 0	0	0	0	8 ± 1	0	0	7 ± 0

Table 4: Antibacterial activity of *T. wightii*

Bacterial Strains	Zone of inhibition (mm): Values = Mean ± Standard Deviation									
	Rhizome				Fronds				Antibiotic	
	PE	EA	M	A	PE	EA	M	A	Methicillin (5 mcg)	Amoxyclav (30 mcg)
K. pneumoniae	0	0	12 ± 0	8 ± 1	0	0	11.33 ± 0.57	8 ± 1	0	0
S. typhimurium	0	0	12 ± 1	9 ± 0	0	0	14.66 ± 0.57	8 ± 0	0	8 ± 0.1
P. aeruginosa	0	0	0	0	0	0	0	0	0	7 ± 1
E. coli	0	0	0	0	0	0	8.66 ± 0.57	0	0	7.66 ± 0.57
L. monocytogenes	0	8.66 ± 0.57	0	0	0	0	0	0	0	6.83 ± 0.28
X. campestris	0	0	12.66 ±0.57	9± 0	0	0	15 ± 1	0	0	7 ± 0

The results of antibacterial activity of various extracts, of both *T. coadunata* and *T. wightii* showed significant effect against the strains tested (Table 3 & 4). Ethyl acetate and methanol extracts of both plants showed remarkable antibacterial effect while, petroleum ether extracts were not effective. Extracts of *T. coadunata* were more sensitive to *S. typhimurium, E. coli* and *X. campestris* whereas the extracts of *T. wightii* were effective against *K. pneumoniae, S. typhimurium* and *X. campestris*. While comparing the effect of rhizome and fronds, the extracts of fronds showed considerable inhibition on bacterial growth. The respective pure solvents that were taken as negative control showed no inhibitory action. The antibiotic amoxyclav showed zone of inhibition against the bacterial strains tested whereas methicillin did not show any response (Figures 1 & 2). Based on the results of antibacterial evaluation *T. wightii* has more potential phytoconstituents than *T. coadunata*.

Phytochemical studies carried out by Muraleedharannair *et al*., (2012) on extracts of *Adiantum caudataum, A. latifolium, A. lunulatum, Christella dentata* and *C. parasitica* confirmed the presence of carbohydrates, steroids, tannins, saponins, carboxylic acid, coumarins, xanthoprotein and phenolic compounds. Similarly, the present study also revealed the presence of various phytochemicals in *T. coadunata* and *T. wightii*. It has been found that the fern extracts were effective against both gram positive and gram negative bacteria (Manickam and Irudayaraj, 1992). Recent studies reported that ethyl acetate and methanolic extracts of *Pityrogramma calomelanos* of Order Polypodiales, is effective against pathogenic bacterial strains like *S. typhimurium*, *P. aeruginosa*, *E. coli* and *X. campestris* (Thomas *et al*., 2020). The present findings also indicate the efficiency of ethyl acetate and methanolic extracts of *T. coadunata* and *T. wightii* against the bacterial strains tested. The various phytoconstituents present in the plant extracts has provided an antimicrobial potential to them. Therefore, this study will further initiate the way for isolation and characterization of bioactive compounds from both the ferns.

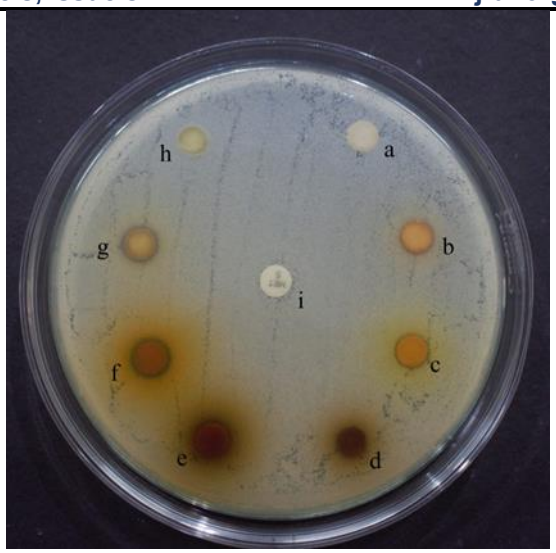

Fig. 1: Antibacterial activity of *T. coadunata* against *S. typhimurium* (a- Petroleum ether extract of rhizome; b- Ethyl acetate extract of fronds; c- Methanol extract of rhizome ; d- Aquoeous extract of rhizome; e- Aquoeous extract of fronds ; f- Methanol extract of fronds ; g- Ethyl acetate extract of fronds; h- Petroleum ether extract of fronds; i- Methicillin)

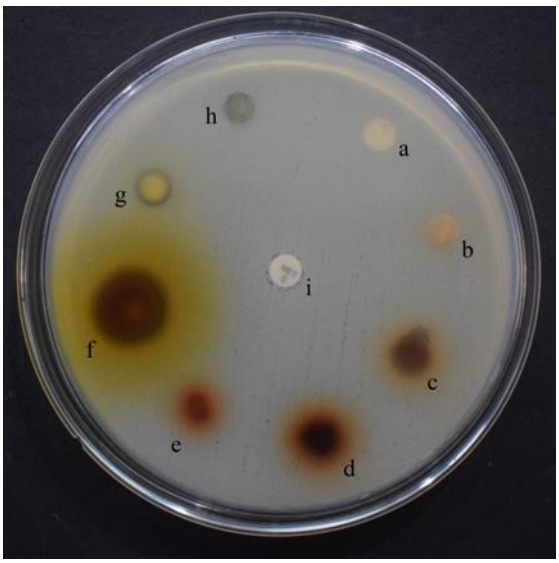

Fig. 2: Antibacterial activity of *T. wightii* against *X. campestris* (a- Petroleum ether extract of rhizome; b- Ethyl acetate extract of fronds; c- Methanol extract of rhizome ; d- Aquoeous extract of rhizome; e- Aquoeous extract of fronds ; f- Methanol extract of fronds ; g- Ethyl acetate extract of fronds; h- Petroleum ether extract of fronds; i- Methicillin)

IV. CONCLUSION

This study was promising in identifying two fern species which could act as a reservoir of antibacterial phytoconstituents. The present work is a preliminary corroboration of antibacterial efficacy of *T. coadunata* and *T. wightii*, which may lead to the purification and isolation of novel phytochemical compounds.

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