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PHYTOCHEMICAL AND NUTRITIONAL BENEFITS OF WILD ENDEMIC EDIBLE TUBER: *THERIOPHONUM FISCHERI* SIVAD.

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ABSTRACT

The herbal species *Theriophonum fischeri* Sivad. belonging to the family Araceae is a wild edible tuberous plant, endemic to Southern Western Ghats holding various traditional cures. Hence the present investigation was encouraged with the phytochemical, nutritional and anti-microbial activity of tuber and leaves of the species. The nutritional analysis of the species resulted with the carbohydrate holds the front chair followed by protein and with traces of trypsin inhibitor. The extractive yield, phytochemicals and antioxidant activity were at the peak for the ethanolic tuber extract adding the significant antimicrobial activity against the bacterial strain *Pseudomonal aeruginosa* and *Klebsella pneumoniae*. The High Performance - Liquid Chromatography (HPLC) analysis confirmed that plant parts possess catechin, gallic acid and quercetin which are proved to hold the above cure properties decades ago. On further investigation, much more eminent properties are to be relieved and hence the endemic species is to be conserved in its native in regard of its therapeutic properties.

KEYWORDS: *Theriophonum fischeri*, Nutritional, Phytochemical, Antimicrobial, HPLC, Western Ghats.

INTRODUCTION

More than a few plant species possess a diverse chemical constituent that are traditionally exploited in the management of diseases. Many of the drug preparations were based on the uses of these agents in custom medicine. India has a rich flora of herbal plants and ancient

medicinal systems which are of eras old. The stable progress of the global market for traditional medicines recommend that increased research utilizing developed research technologies may be essential to improve our understanding of the composition, efficacy, safety and tolerability of traditional medicines. There are more than thousand known and more unknown phytochemicals. Researchers concluded that these phytochemicals not only protect and develop the plant species themselves but also has the potential to cure various diseases of other organisms including human beings. The family Araceae is emphasised for their traditional usages since ages. Species of the family has nutritional and antinutritional benefits, antioxidant, hepatoprotective, antimicrobial, antitumor and used as phytomedicine.[1] *Theriophonum fischeri* belonging to the family araceae, endemic to Southern Western Ghats is such a traditionally used medicinal plant of less notice. The species like *Colocasia esculenta*, *Amorrphophallus paeoniidolius*, *Monstera deliciosa*, etc. belongs to the same family. *T. fischeri* possesses traditional medicinal cures against piles, body pain, skin troubles, wounds[2], etc. Hence the present study aims at the clinical evaluation of the species in cure of the certain ailments.

MATERIALS AND METHODS

The fresh plant parts (leaves and tubers) of *Theriophonum fischeri* were collected from Sirumalai Hills of Dindugal district during the month of November, 2014. The specimen was authenticated from Botanical Survey of India Southern Division, Coimbatore (Ref.No. BSI/SRC/5/23/2014/Tech./116). The parts are well cleaned and shade dried, powdered and stored -20°C.

Extract recovery percentage

25g of powdered plant materials were subjected for successive solvent extraction with petroleum ether, chloroform and ethanol. The extracts were filtered and concentrated to dryness and were stored at -20°C until used directly for further analysis. The percentage yield (recovery) of evaporated plant extracts was calculated.

Nutritional and anti-nutritional evaluation

Carbohydrates, free amino acids, starch and fatty acids were evaluated by Sadasivam and Manickam, 1996[3] and proteins by Lowry *et al*., 1996.[4] The anti-nutritional contents of the samples were determined using the standard methods: Phytic acid[5] trypsin inhibitor activity[6] with some modification, Oxalate.[7]

Qualitative and quantitative phytochemical analysis

The extracts were analysed qualitatively for the presence of phyto-chemicals.[8,9] The total phenolic and tannin contents were determined according to Makkar, 2003[10]; the total flavonoid content of the extracts was quantified using the procedure put forth by Zhishen *et al*., 1999.[11]

***In vitro* antioxidant activity**

The antiradical activity of the species was assessed using DPPH$^{\bullet}$ method[12] and Reducing power assay[13] and the outcomes were matched with the activities of rutin, quercetin, BHA and BHT. The former activity was expressed as IC_{50}, a lower IC_{50} value indicates greater antioxidant activity; the latter, a higher absorbance indicates a higher reductive capability.

Further the Phosphomolybdenum assay[14] and Ferric reducing antioxidant power (FRAP) assay[15] were carried out and the results were calculated in ascorbic acid, Ferric sulphate equivlents. Superoxide radical scavenging activity[16] was also performed. The sample concentration providing 50% inhibition (IC_{50}) under the assay condition was calculated from the graph of inhibition percentage against sample concentration.

Antimicrobial activity

Test Microorganisms

The bacterial strains *Stphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonal aeruginosa and Escherichia coli* and the fungal strain *Candida albicans* were obtained from Clinical Biotechnology Laboratory, Microbial Biotechnology, Bharathiar University and were maintained on nutrient agar plates and Sabouraud dextrose agar plates at 4°C respectively. The antimicrobial activity of the plant *T. fischeri* was carried out using the well diffusion method described by Dulger and Gonuz, 2004.[17]

Preparation of standard

500 mg of Gentamycin was dissolved in 5 ml of distilled water for the antimicrobial assay. 12 g of nutrient agar was dissolved in 250 ml of distilled water in a conical flask. The nutrient agar was poured into sterilized Petri dishes after sterilization. After solidification, wells were made sing a sterilized cork borer and microorganisms were introduced. The dissolved antibiotics solution was poured into the wells using a dropping pipette after which the Petri dishes were incubated for 24 hours at 37°C. The inhibitory zones in millimetre were measured and recorded.

High Performance Liquid Chromatography (HPLC)

The chromatographic measurements were performed using Shimadzu HPLC system (Japan) equipped with a UV detector (model : 2998) and a reversed phase column (Luna, φ 4.6mm x 250mm, 5 µm). The mobile phase consists of degassed solvent A (3% acetic acid in water) and solvent B (3 : 50 : 47 :: acetic acid : acetonitrile : water). Quantification was performed by independently for ec extracts from the standard graphs using the formula:

$$C\,(sa) = \frac{C\,(st) \times A\,sa)}{A\,(st)}$$

Where, C (sa) is concentration of compound in sample; C (st) is concentration of standard; A (sa) is area of peak I sample and A (st) is area of peak in standard.

Statistical analysis

For quantification of phytochemicals, nutrients and anti-nutrients, *in vitro* antioxidant and *in vitro* antimicrobial activity of the extract, the results were recorded as mean ± standard deviation (SD) (n = 3).

RESULTS AND DISCUSSIONS

Extract yield percentage

The extract yield represents the amounts of active components extracted from plant species. The extractive yield of the plant *Theriophonum fischeri* after successive solvent extraction resulted with a higher yield of 7.84% and 0.96% in the ethanolic extract of leaf and tuber respectively (Fig.1). The higher extractive yield of leaf and tuber parts of the species supports that the active components were generally higher in polar solvents than in non-polar solvents, suggesting that the former solvents were more suitable for extracting the phytoconstituents from plants.[18]

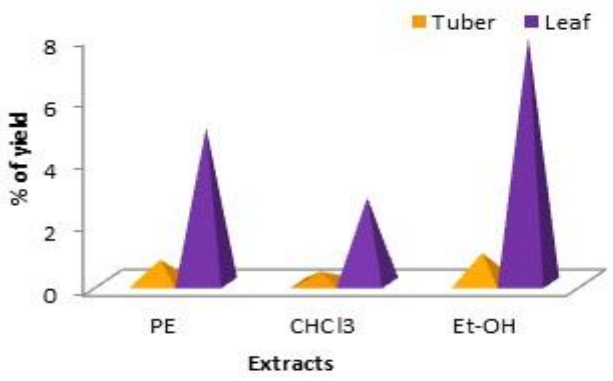


Fig 1: Percentage yield of *T. fischeri.*

Nutritional and anti-nutritional evaluation

The nutritional evaluation of *T. fischeri* tuber came up with a high content of carbohydrate (34.32g/100g), followed by protein (31.65 g/100g) and starch (24.29 g/100g) (Table 1). The elevated carbohydrate, protein and starch in *T. fischeri* tuber (Table 1) add the nutritional; benefits to the mankind. The protein free amino acid and free fatty acid composition are a boon for the edible food. The estimated carbohydrate content of *T. fischeri* is higher than the wild tuber *Colocasia esculenta* which accounts for 26.98%.[19]

Table 1: Nutritional and anti-nutritional composition of *T. fischeri* plant parts.

S.No.	Parameters	Amount *	
		Tuber	Leaf
Nutritional analysis			
1	Carbohydrates (mg GU equivalents/g sample)	34.32±0.1	26.14±0.2
2	Starch (mg GU equivalents/g sample)	24.29±0.3	9.1±0.1
3	Protein (mg BSA equivalents/g sample)	31.65±0.2	17.26±0.2
4	Free amino acids (mg LE equivalents/g sample)	0.14±0.1	0.11±0.2
Anti-nutritional analysis			
1	Trypsin inhibitor (TIU/mg)	0.32±0.001	BDL
2	Phytic acid (%)	1.28±0.001	BDL
3	Cyanogen (mg/l)	0.07±0.001	0.02±0.001
4	Oxalate (%)	0.37±0.001	0.11±0.001

*Values are mean of three independent analysis ± SD (n=3); GU: Glucose. BSA: Bovine Albumin Serum, LE: leucine, TIU: Trypsin inhibitory unit, SD: Standard Deviation.

Among the anti-nutritional factors of *T. fischeri* tuber analysed, the cyanogen (0.02mg/100g) exhibited a minimum amount followed by oxalate (0.27 mg/100g (Table 2). The anti-nutritional factors such as cyanogen, oxalate, phytic acid and tripsin inhibitor found in least amount in *T. fischeri* tuber (Table 1), eliminates the risks on toxicity of the species. It is noted that Cassava, Cocoyam and Yam contain 624mg, 588 mg and 637 mg of phytate per 100g of the samples respectively. The high content of calcium oxalate crystals of about 780 mg per 100 g in some species of Cocoyam, Colocasia and Xanthosoma has been implicated in the acidity and irritation caused by them.[20] Soluble oxalates are generally detoxified in the digestive tract, though if ingested in large quantities they may be absorbed and bind with blood to form blood calcium, thus causing hypocalcaemia. Further it causes renal failure and cause risks to Central Nervous System.[21] Although the high anti-nutrient composition is a potential health risk and they are potent human poison, toxic plant parts are used in medical treatments, usually within the sector of green/alternative medicine. Hence the species exhibits a notable anti-nutritional property that benefits the human physique.[22]

Qualitative and quantitative phytochemical analysis

The bioactive properties in the plants are generally ascribed to the presence of plant secondary metabolites which could have beneficial or adverse effects. It is supposed that most of the known secondary metabolites are to be engaged in plant chemical defense systems, which are formed all over the millions of years during which plants have co-existed competing with their attackers and thus represent rational medicines which can be used to treat a wide range of health disorders, diseases and infections. The presence of major secondary metabolites such as alkaloids, flavonoids, phenols, saponins, tannins and terpenoids were attempted and depicted in Table 2. Among the various solvent types examined, ethanol extracts of leaf and tuber of the species along with the chloroform extract of the tuber indicated the presence of secondary metabolites such as alkaloids, flavonoids phenols, saponin, tannin and terpenoids where, they exhibit properties such as nutritional, antioxidant, anti-inflammatory, antimicrobial, antitumor, anti-proliferative anti-analgesic, anti-ulcer, against neuro-degenarative, Alzheimer's, cardio vascular diseases, etc.[23]

Table 2: Qualitative phytochemical analysis of *T. fischeri* plant parts.

Parts	Extracts	Secondary metabolites*					
		Alkaloids	Flavonoids	Phenols	Saponins	Tannins	Terpenoids
Leaf	PE	-	-	-	++	-	-
	CHL	+++	++	++	+	-	+
	ETH	++	+++	+++	+	+	-
Tuber	PE	+	-	-	+	+	+
	CHL	++	++	++	++	++	+
	ETH	++	+++	+++	+	+	++

PE- Petroleum Ether; CHL- Chloroform; ETH- Ethanol

* +++: highly present, ++: moderately present, +: Low, -: absent.

Plants produce a broad range of bioactive chemical compounds so called secondary metabolism. Plants with potent bioactive compounds are often characterised as both poisonous and medicinal, and a beneficial or an adverse result may depend on the amount eaten and the context of intake. For typical food and feed plants with bioactive compounds with less pronounced effects, the intakes are usually regarded as beneficial.[24]

Metabolites present an array of solubility in solvents with different polarity. Phenolics, tannins and the flavonoids are groups of secondary metabolites which are synthesized by plants; they have been indicated to have several biological activities such as antioxidant, anti-allergic, antimicrobial, anti-aging, anti-diabetic, anti-mutagenic, anti-carcinogenic and anti-

inflammatory.[25] The phenolic, tannin and flavonoid content of *T. fischeri* tuber were estimated. The ethanolic extracts of the tuber and leaf exhibited the higher phenolics (13.65 and 10.29 g GAE/100 g of the extract), tannins (9.48 and 2.63 g TAE100g of the extract) and flavonoid (10.16 and 7.66 g RE/100g extract) of which the former took over the latter (Table 3). These reviews support the vital role of the secondary metabolites in the study species ie., phenolics, tannin and flavonoids (Table 4) in ethanolic extract of *T. fischeri* leaf and tuber as a benefit for the cure against different diseases. It is presumed that the correspondingly higher amounts of flavonoids in polar solvents might be due to the relative composition of water-soluble compounds such as flavonols. The genus is marked with the presence of flavone *C*-glycosides, flavonols, flavones, anthocyanins, proanthocyanidins, kaempferol and quercetin 3-(6-arabinosylgalactoside), kaempferol 3-xylosylgalactoside, etc.[26]

Table 3: Quantitative phytochemical analysis of *T. fischeri* plant parts*.

S.No.	Plant Parts	Extracts	Total Phenolics (g GAE/100g extract)#	Tannins (g TAE/100g extract)#	Total Flavonoids (gRE/100g extract)@
1	Tuber	Petroleum ether	8.40 ±0.05^{c}	5.91±0.50^{b}	2.49±0.54^{c}
2		Chloroform	8.94±0.65^{c}	7.22±0.34^{a}	1.72±0.46^{c}
3		Ethanol	**13.65±0.48^{a}**	**9.48±0.26^{a}**	**10.16±0.07^{a}**
4	Leaf	Petroleum ether	9.49±0.75^{c}	1.76±1.13^{c}	7.22±0.38^{a}
5		Chloroform	10.10±0.07^{b}	0.28±0.11^{c}	4.17±0.26^{b}
6		Ethanol	**10.29±0.46b^{b}**	**2.63±0.41^{c}**	**7.66±0.07^{a}**

*Values are mean ± SD of three independent experiments. Values not sharing a common letter in a column are significantly different (P<0.05). # Values expressed as mg GAE/g extract; @ Values expressed as mg RE/g extract

In-vitro Antioxidant activity

As a common factor in pathogenesis of chronic degenerative disease is the involvement of oxidative stress, it is hypothesised that this healthy dietary pattern may dampen oxidative stress and thereby reduces the risk of diseases. Oxidative stress is a type of chemical stress induced by the incidence in our body of irregular quantities of ROS. This condition may be due to an increased production of ROS and/or of a decreased efficiency of the antioxidant defence systems. Thus, when the critical balance between generation of ROS and the antioxidant defence is unfavourable, oxidative damage can accumulate.[27]

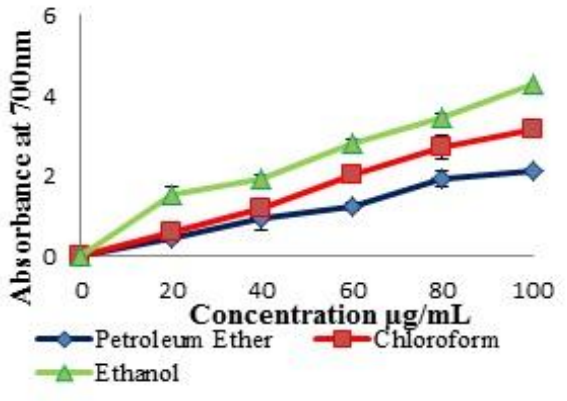
The antioxidant activity of *T. fischeri* leaf and tuber was analysed by the DPPH$^{\bullet}$, phosphomolybdenum assay, FRAP, Reducing Power and Superoxide radical scavenging

assays where, the tuber and leaf extracts expressed efficient scavenger of superoxide radical generated in riboflavin- NBT- light system *in vitro*. Among the assays analysed, higher antioxidant activity was seen in the ethanolic extract of tuber followed by ethanolic extract of leaf. The DPPH$^{\bullet}$ Radical Scavenging activity of the ethanolic extract of *T. fischeri* tuber (25.664 IC_{50} µg/mL) and leaf (29.981 IC_{50} µg/mL) revealed higher activity and the chloroform exhibited minimal activity (Table 5). The elevated Phosphomolybdenum (2.456 g AAE / 10 g of the extract) and FRAP activity (85.059 m mol Fe (II) E/mg extract) was seen in the ethanolic tuber extract and Superoxide radical scavenging activity (24.887 IC_{50} µg/mL and 26.166 IC_{50} µg/mL) in ethanolic leaf extract (Table 4). The ethanolic extract of the tuber of the species exhibits the higher reducing power (5.021 g AAE/100g extract) than the other plant part and extracts (Fig. 2a-c).

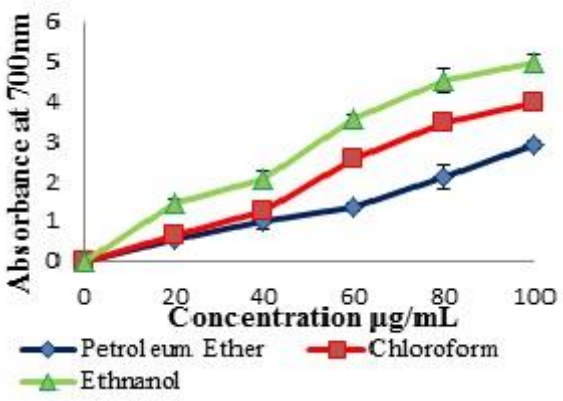
Table 4: Antioxidant activity of *T. fischeri* plant parts*.

Parts	Extracts	DPPH (IC_{50} µg/mL)	$O_2^{\bullet-}$ (IC_{50} µg/mL)	FRAP (m mol FE (II) E/mg extract)	Phospho-molybdenum (g AAE/100g of extract)
Leaf	PE	117.442±0.61^{c}	44.170±0.08^{b}	0.930±0.44^{c}	0.474±0.08^{c}
	CHL	108.020±0.23^{c}	33.325±0.41^{a}	1.316±0.53^{c}	0.832±0.15^{c}
	ETH	**29.981±0.22^{a}**	**26.166±0.15^{a}**	**67.035±2.45^{a}**	**2.456±0.03^{a}**
Tuber	PE	86.599±0.48^{b}	86.134±0.91^{c}	0.965±0.37^{c}	0.922± 0.11^{c}
	CHL	53.46±0.1^{ab}	52.710±0.25^{b}	1.558±0.18^{c}	1.558±0.18^{b}
	ETH	**25.664±0.27^{a}**	**24.877±0.32^{a}**	**85.059±0.29^{a}**	**2.059±0.29^{a}**
Standard	Rutin	15.8±0.1^{a}	18.8±0.01^{a}	-	-
	Quercetin	20.7±0.1^{a}	23.0±0.07^{a}		
	BHA	21.4±0.1^{a}	26.4±0.51^{a}		
	BHT	34.7±0.3^{a}	39.5±0.63^{a}		
	-	-	-	Equvalent to $FeSo_4$	Equvalent to Ascorbic acid

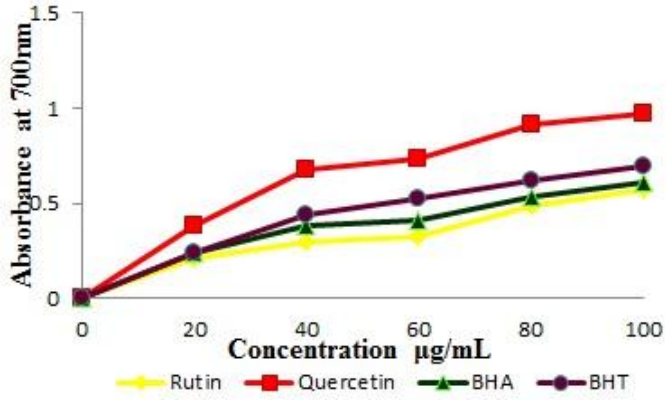
*Values are mean ± SD of three independent experiments. Values not sharing a common letter in a column are significantly different (P<0.05).



a) Reductive capability of *T. fischeri* leaf.



b) Reductive capability of *T. fischeri* stem.



c) Reductive capability of standards.

Fig. 2: (a-c). Antioxidant activity through reducing power assay.

The results were supported to be so due the enhanced activity of ethanol extracts may be due to the polar nature and extracting ability of phenolic compounds from the parts under study.[28] The probable mechanism of the superoxide radical scavenging activity of the plant may be due to the active principles in the plant extracts which may eliminate the superoxide anion radicals which are generated through photo-illumination process. The plant that experiences stress (from for example sunlight, drought, microorganisms) they speed up their antioxidant production and hence, stressed plants therefore contain the most antioxidants.[29] Hence the endemic study species fits the property both theoretically and experimentally.

Antimicrobial activity

Infectious diseases are the major cause of morbidity and mortality worldwide. The use of plant extracts with known antimicrobial properties can be of great significance for therapeutic treatment. Recently there has been considerable interest in the use of plant material as an

alternative method to control pathogenic microorganism and many components of plants products have been shown to be specially targeted against resistant pathogenic bacteria.[30]

Table 6: Anti-microbial activity of ethanolic extract of *T. fischeri* tuber*.

S.No	Pathogen	Zone of inhibition (mm)		
		Control (Gentamycin)	Ethanolic Extract	
			Tuber	Tuber
1	*Staphylococcus aureus*	25.07±0.25	18.02±0.41	14.51±0.25
2	*Escherichia coli*	24.00±0.74	14.21±0.15	12.67±0.28
3	*Klebsella pneumoniae*	22.21±0.42	**21.11±0.25**	15.19±0.75
4	*Pseudomonas aeruginosa*	26.05±1.23	**24.27±0.34**	**23.35±1.22**
5	*Candida albicans*	24.62±0.35	12.84±0.73	5.51±0.69

* Values are mean ± SD of three independent experiments.

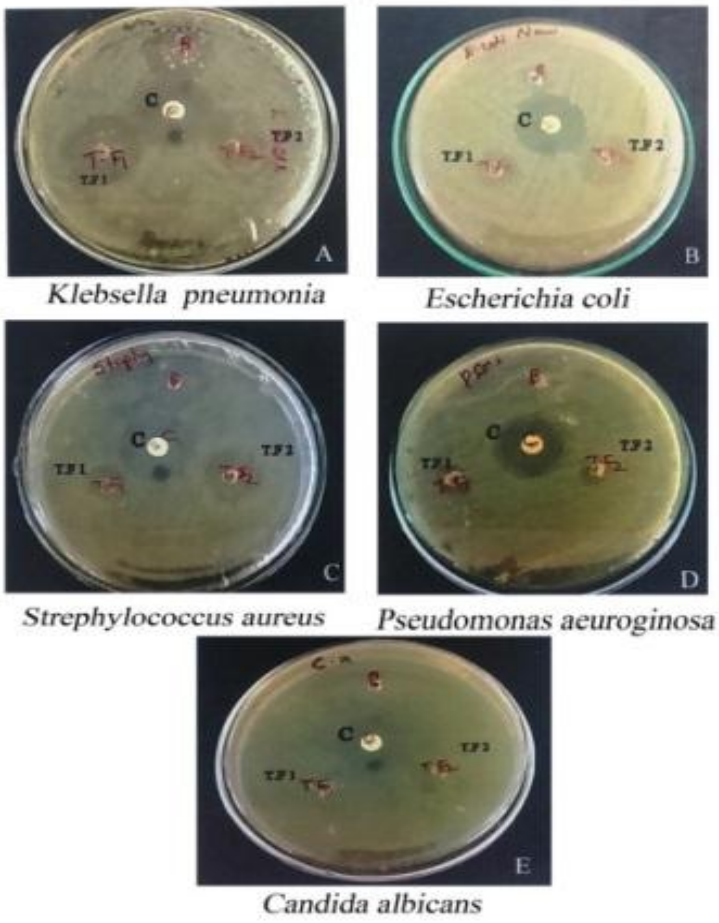


Fig 3: Anti-microbial activity of ethanol extract of *T. fischeri* tuber.

The antimicrobial activity of ethanolic extract of *T. fischeri* tuber and leaf showed a preferable antibacterial activity where, the tuber exhibited a higher activity comparing the leaf against *Pseudomonas aeruginosa* (24.27±0.34 nm) and *Klebsella pneumonia*

(21.11±0.25) which is comparable to the standard gentamycin (Table 5; Fig. 3). The results of the study support the folklore claim along with the development of new antimicrobial drugs from both the plants.

High Performance Liquid Chromatography (HPLC)

Bioactive herbal components are broadly used both in food and feed and cover different categories and products. HPLC fingerprinting is the best way for chemical characterization, and therefore this study also established HPLC fingerprint for the active phenolic compounds that can act as antioxidant, antifungal, antibacterial, anti-inflammatory and anti-tumour agents.[31] Much attention has been focused on the protective biochemical function of naturally occurring antioxidants in biological systems.

The results of the HPLC for ethanolic extract of *T. fischeri* leaf and tuber led to the isolation of 3 compounds i.e., gallic acid, catechin and quercetin with a duration 64.294 min. The type of compound that appeared in the peak numbers 1, 2 and 3 of ethanolic extract of *T. fischeri* tuber and the peaks 1, 3 and 5 of ethanolic extract of *T. fischeri* leaf which investigated at 3.617 min, 9.055 min and 62.680 min and 4.285 min, 10.208 min and 64.294 min respectively are similar to the standard gallic acid, catechin and quercetin respectively. (Table 6; figure 4, 5).

Table 7: Area of percentage in chromatogram of ethanolic extract *T. fischeri* leaf and tuber.

Peak Name	RT* (mins)	Area	% Area	Height	Concentration
Tuber					
Gallic Acid	3.617	215747	3147	3.367	1.80
Catechin	9.055	4233740	44279	66.07	20.90
Quercetin	62.680	1957626	23242	30.55	3.40
Leaves					
Gallic Acid	4.285	80290	0.9	1409	0.67
Peak 2	8.616	183283	2.26	3333	-
Catechin	10.208	815687	10.07	22138	4.00
Peak 4	29.853	6812821	84.13	15369	-
Quercetin	64.294	205689	2.54	4552	0.35

*Retention Time

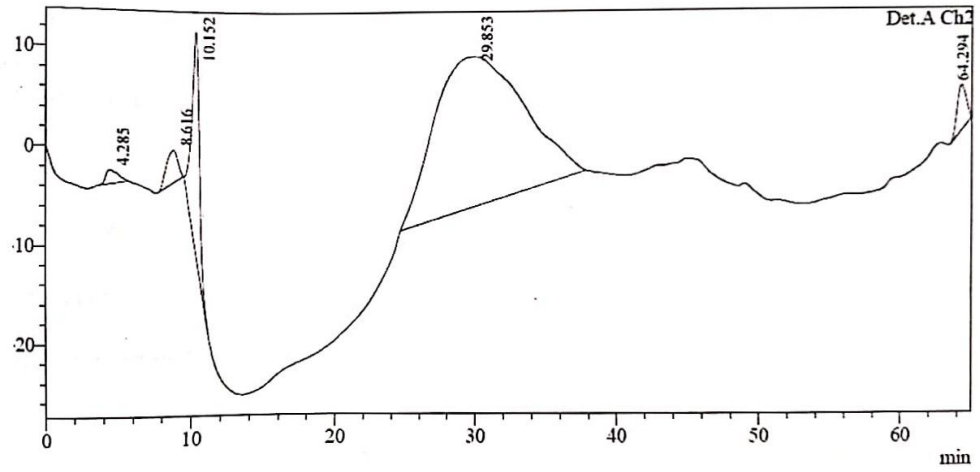


Fig. 4: HPLC chromatogram of *T. fischeri* leaf.

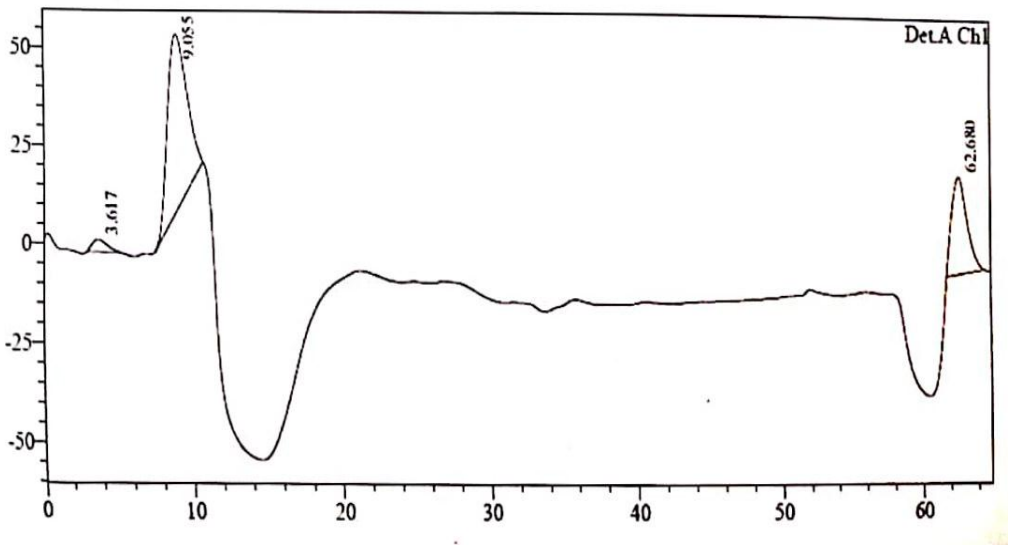


Fig. 5: HPLC chromatogram of *T. fischeri* tuber.

The peak in *T. fischeri* tuber shows that one of the majority compounds is catechin, a natural phenol (20.9 µg/mg extract) followed by quercetin (3.4 µg/mg extract) and gallic acid (1.80 µg/mg extract) present in high concentration. Identically, the ethanolic extract of leaf revealed the higher concentration of catechin (4 µg/mg extract) followed by gallic acid (0.67 µg/mg extract) and quercetin (0.35 µg/mg extract) (Table 9).

Table 9: Concentration of identified compounds in *T. fischeri* leaf and tuber.

S.No.	Name of the compound	Ethanolic extract of Leaf (µg/mg extract)	Ethanolic extract of tuber (µg/mg extract)
1	Gallic Acid	0.67	1.80
2	Catechin	4.0	20.9
3	Quercetin	0.35	3.4

These compounds are being with the vital role as agent for cure of various diseases such as diabetes, oxidative stress mediated diseases, tumor, coronary heart diseases, arthritis, neuro-degenerative disorders, osteoporosis, ulcer, prostatitis, microbial infections, etc.[32-34]

CONCLUSION

From the results of the present study, it is evident that the ethanolic extract of tuber contains phenolic compounds catechin, quercetin and gallic acid besides its other phyto-constituents which is also shown by HPLC analysis and other experiments. A positive and significant correlation existed between antioxidant activity and total phenolics. Therefore, this investigation suggests that the ethanolic extract of tuber of *Theriophonum fischeri* might be a potential source of natural drug against microbes and oxidation mediated diseases which might be an alternate to synthetic drug available in the market. Hence, its beneficial effect on animal and human health may be derived from its antioxidant properties to afford protection against various diseases as mentioned above.

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AUTHOR CONTRIBUTION

Both the authors contributed in the fields of concept and manuscript correction of the research work. The first author contribution extends with the design, literature review, experimental analysis, manuscript preparation, editing and review.

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