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ANTIMICROBIAL, ANTHELMENTIC, ANTIOXIDANT AND PHYTOCHEMICAL INVESTIGATION OF *GLOCHIDION TOMENTOSUM* DALZ.AN MEDICINAL TREE

KEY WORDS: Antimicrobial, Antioxidant, *Glochidion tomentosum*, Antioxidant, Phytochemical screening

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ABSTRACT

In the present study, we made an effort to screen the bioactive compounds from endangered medicinal tree *Glochidion tomentosum* Dalz. collected from Western Ghats regions of Karnataka. Preliminary phytochemical analysis of soxhlet extracts in bark of *G. tomentosum* revealed the presence of tannins, terpenoids, alkaloids, glycosides, steroids and absence of flavonoids and saponins. The carbohydrate content (59.9 %) and nutritive value (cal/100 g) was found to be greater than 300 cal/100 g. Macro nutrients like phosphorus and potassium was found to be higher content. In case of microelements viz., Fe, Mn, Zn and Cu are found to possess high concentration.The result of antibacterial activity of *G. tomentosum* was determined against the panel of 13 pathogenic bacteria. Among bacteria, more inhibition was recorded in case of *Pseudomonas aeruginosa* by extracts followed by *Escherichia coli* and *Staphylococcus aureus*. *P. aeruginosa* was inhibited at low concentration (0.35 mg/ml) followed by *E.coli* (0.40 mg/ml) and *S. aureus* (0.50 mg/ml) by ethanol extract of *G. tomentosum.* Minimum inhibitory concentration of *G. tomentosum* was found to be 0.30, 0.30 and 0.40 mg/ml for *E. coli*, *P. aeruginosa* and *S. aureus* respectively. In case of *G. tomentosum* the anthelmintic effects were comparable with that of 1% piperazine citrate with higher doses namely 30mg/ml and higher. Overall, anthelmintic potential was higher in ethanol extract than petroleum ether extract. The ethanol extract of *G. tomentosum* have shown concentration dependent radical scavenging activity. It showed 89.41 % of inhibition at 1 mg/ml concentration. The extracts have shown dose dependent scavenging activity. Ethanol extract showed 82.70±1.560 % of inhibition and petroleum ether extract showed 69.31±1.350 % of inhibition at 60 µg/ml concentrations.

INTRODUCTION

Medicinal plants and its constituents play an important role in the treatment of localized and generalized infections [1]. According to World Health Organization majority of the people living in the developing countries depends on the traditional system of medicine for the treatment of various diseases [2]. Medicinal plants are the active components of Ayurveda, Unani and Siddha systems of medicine. It has been estimated that 15-30% of higher plant species are used medicinally [3].

At present, plant medicine comes from plant extraction, has occupied nearly 30 percent to 40 percent among the thousands of worldwide used pharmaceutical products [4]. Chemical compositions of various medicinal plants are very complex, usually containing many kinds of effective ingredients [5]. Numerous methods, including conventional solvent extraction, steam distillation, sublimation, etc., are known for extracting phytochemicals from plant materials, most based on sequential extraction processes incorporating one or more organic solvents in combination with washing steps [6]. While such methods are useful for extraction and purification of small quantities of phytochemicals for research purposes, they are difficult to scale to commercial through-put volumes because of the problems associated with cost-effectively, safely and completely removing and recovering the organic solvents from the extracts and spent plant materials [7].

The genus Glochidion commonly called as cheese trees or button wood trees consisting of 300 species [8]. The leaves of *G. tomentosum* will be used for treatment of wounds [9]. Leaf paste from this plant used as an ointment for wounds [10]. Other species of *Glochidion* are very well known for their medicinal values and used for the treatment of dysentery, cough itches, eczema, enteritis, stomach ailments and rheumatism [11]. The leaves, fruits and bark of *Glochidion zeylanicum* has been used for itches, cooling, restorative and stomachic [12]. *Glochidion littorale* is used to stop dysentery and to assuage stomach ache [13]. *G. rubrum* is used to heal heamorroids [14]. Several triterpenoids, triterpenoid glycosides and alkaloids are known to be constituents of the plants belonging to the genus *Glochidion* [15]. Neolignan glucoside, glochidioboside, dendranthemoside B and icariside B1, bergenin and benzyl alcohol glucoside, blumeol C glucoside, megastigmane glucosides are some of the chief constituents of *Glochidion* genus [16]. Lignans and triterpenoids are also known to occur in *Glochidion sp.,* [17, 18]. The plants having triterpenoids are the most widely used for the treatment of inflammation and many other life threatening diseases in the traditional medicine of different cultures [19].

Glochidion species are used as food plants by the larvae of some Lepidoptera species including *Aenetuseximia* and *Endoclitadamor* [20] The Nicobarese people have attested to the medicinal properties found in *G. calocarpum*, saying that its bark and seed are most effective in curing abdominal disorders associated with amoebiasis [21]. Novel techniques offers a great opportunity for developing countries those have potential in the development of their herbal medicines as an important industry. The development of herbal product including medicinal plants and essential products and spices for export can help to increase income among farmers, reduce poverty and stimulate entrepreneurship and create a favorable business environment to integrate into the global market place.

2. MATERIALS AND METHODS:

2.1. Collection and identification the plant material:

The bark of *Glochidion tomentosum* was collected from Sakaleshpur, Hassan district, Karnataka, India. Identification of the plant was carried out by Dr.P. Sharanappa and voucher specimen (PS 271/2016) was deposited at Department of Bioscience, P.G. Centre, Hemagangothri, Hassan, Karnataka, India.

2. 2. Extraction of secondary metabolite from bark of *Glochidion tomentosum:*

The bark of *G. tomentosum* was washed, air dried and then powdered (40 mesh size) and the same material used for the different solvent extraction using the soxhlet. The obtained solvent extracts were evaluated for their biological activities [22].

2.3. Determination of Extraction yield:

Different solvent extracts of the bark of G. tomentosumwere collected by using soxhlet extraction method. The extract was filtered through whatman filter paper no. 1 to remove solid particles, if any. The filtered extract was then completely dried in the oven at 40°C and the final constant weight was recorded and calculated [23].

$$Y_{extract} = \frac{m_{extract}}{m_{herb}} \ 100$$

Where, $Y_{extract}$ is the % extraction yield, $_{mextract}$ is the crude extract mass (g) and mherb is the extracted herb mass (g).

2.4. Preparation of sample solution:
Stock solutions of the different fractions of bark of *Glochidion tomentosum* at the concentration of 1000μg/ml were prepared using DMSO as a solvent. DMSO was sterilized by filtration using filter paper (pore size 0.2 microns). Further dilution was made by sterile DMSO to get concentration of 200 μg/ml of each extract [24].

2.5. PHYTOCHEMICAL SCREENING OF THE BARK OF *G. TOMENTOSUM.*
2.5.1. Proximate Analysis: Various proximate parameters namely moisture, ash, crude fiber, crude fat, protein and carbohydrate content were analyzed in the dried and powdered bark of *G. tomentosum* [25].

2.5.2. Determination of Elemental Composition of bark of *G. tomentosum.*
The concentration of macro elements namely Potassium (K) and Phosphorus (P) and microelements namely Copper (Cu), Manganese (Mn), Iron (Fe) and Zinc (Zn) was estimated using atomic absorption spectrometer [26].

2.5.3. Qualitative analysis of Phytochemicals*:*
Phytochemical tests are done in plant extracts for the recognition of presence of different chemical constituents such as; alkaloids, glycosides, phenolic compounds, flavonoids, essential oils, carbohydrates, proteins, steroids, saponin glycosides, tannins and other substances which are accountable for the biological activity [27].

2.6. Antimicrobial Activity of ethanol and petroleum ether extract of bark of *G. tomentosum.*
The efficacy of the bark extract of *G. tomentosum* was evaluated against the panel of 13 bacterial pathogens viz., *S.aureus* (NCIM-2079), *P. aeruginosa* (MTCC-90), *E. coli* (MTCC-1610), *B. subtilis* (NCIM -2063), *V. parahaemolytica* (MTCC-451), *B coagulase* (MTCC-492), *A. baumanni* (NCIM-5152), *S. typhi* (MTCC-734), *S. flexineri* (MTCC-1457), *Micrococcus spp.* (NCIM-2913), *B. megatarium* (MTCC-4912), *S. sonii* (MTCC-2959), *K. pneumonia* (MTCC-109) [28].

2.7. Anthelmentic Activity of ethanol and petroleum ether extract of bark of *G. tomentosum.*
Activity was performed as per the method of Neogi and Nayak, 1958.The relative biological activity was evaluated on adult Indian earth worm *Pheretima posthuma* [29]. From three different concentrations extract (10,100,200 mg/ml in saline) was treated for the study of anthelmintic activity (paralysis & death), six worms (same type) were placed in it. Observations were made for both type of worms and the time taken to cause paralysis and death of the individual worms calculated. Mean time for paralysis & death time was recorded; piperazine citrate (10 mg/ml) was used as reference standard [30].

2.8. Free radical scavenging activity of ethanol and petroleum ether extract of bark of *G. tomentosum.*
The free radical scavenging activity (antioxidant capacity) of the test samples was assessed by 1,1-diphenyl-2-picrylhydrazyl (DPPH). Here, 2.0 ml of a methanol solution of the sample (test sample/ standard) at different concentration were mixed with 3.0 ml of a DPPH methanol solution (20 μg/ml). After 30 min of reaction at room temperature in dark place the absorbance was measured at 517 nm by UV spectrophotometer by using methanol as blank. Inhibition of free radical DPPH in percent (I %) was calculated as follows:
(I %) = (1 - Asample/Ablank) × 100
Where Asample is the absorbance of the sample and Ablank is the absorbance of the control (containing all reagents except the test material). Sample concentration providing 50% inhibition (IC50) was calculated from the graph plotted with inhibition percentage against sample/standard concentration.

3. RESULTS AND DISCUSSION:
3.1. Phytochemical screening of bark extract of *Glochidion tomentosum*:
Preliminary phytochemical analysis revealed the presence of tannins, terpenoids, alkaloids; glycosides were detected in ethanol extracts of bark of ***G. tomentosum*** shown in Table-3.1.

Table-3.1: Phytochemical constituents detected in bark of *Glochidion tomentosum*.

Metabolite	*Glochidion tomentosum*
Tannins	+
Flavonoids	-
Alkaloids	+
Steroids	-
Glycosides	+
Saponins	-
Terpenoids	+

3.2. PROXIMATE ANALYSIS:
The proximate composition of G. tomentosum is shown in Table 3.2. Itwas found that, G. tomentosum to possess high moisture content 15.2 %. Ash content was found to be 8.9 %. Advantage of G. tomentosum lies in its comparatively high crude fiber (12 %) and protein content (16.2 %). 53.2 % carbohydrate content was observed and total Nutritive value (Cal/100 g) was found to be greater than 300 cal/100 g.

TABLE-3.2.PROXIMATE COMPOSITION OF BARK EXTRACT OF *GLOCHIDION TOMENTOSUM*

Proximate parameter	*Glochidion tomentosum*
Moisture (%)	15.2
Ash (%)	8.9
Fibre (%)	12.0
Protein (%)	16.2
Fat (%)	6.5
Carbohydrate (%)	53.2
Nutritive value (Cal/100g)	336.1

TABLE-3.3: ELEMENTAL COMPOSITION OF *GLOCHIDION TOMENTOSUM*

Plant	Elemental composition					
	Macro elements (%)		Micro elements (ppm)			
	P	K	Fe	Mn	Zn	Cu
G. tomentosum	0.079	0.090	15937	152.4	81.5	142

Elemental composition of Glochidion tomentosum tested showed the presence of phosphorus and potassium content. In case of microelements, Fe, Mn, and Zn were rich and found to possess high concentration of Cu as shown Table-3.3.

3.3. ANTIMICROBIAL ACTIVITY OF *G. TOMENTOSUM*
Table-3.4: Antibacterial activity of *Glochidion tomentosum* and standard against pathogenic bacteria.

Test bacteria	Zone of inhibition in cm			
	Ethanol extract	Pet ether extract	Control	Standard
S. aureus (NCIM-2079)	2.4	2.2	-	3.4
P. aeruginosa (MTCC-90)	2.5	2.4	-	3.2
E. coli (MTCC-1610)	2.8	2.3	-	3.0
B. subtilis (NCIM -2063)	2.6	2.3	-	2.9
V. parahaemolytica (MTCC-451)	0.4	0.9	-	2.5
B. coagulase (MTCC-492)	1.5	1.9	-	2.5
A. baumanni(NCIM-5152)	0.8	1.2	-	2.0
S. typhi(MTCC-734)	1.1	1.9	-	2.8
S. flexineri(MTCC-1457)	0.5	0.9	-	2.2
Micrococcus spp. (NCIM-2913)	1.5	1.8	-	3.1
B. megatarium (MTCC-4912)	1.9	2.4	-	3.5
S. sonii(MTCC-2959)	0.6	0.8	-	2.5
K. pneumonia (MTCC-109)	1.5	2.3	-	3.5

The result of antibacterial activity of ethanol and petroleum ether extract in bark of *Glochidion tomentosum* is shown in Table-3.4. Results were recorded as presence or absence of zones of inhibition around the well. The inhibitory zone around the well indicated the absence of bacterial growth and it as reported as positive and absence of zone as negative. The inhibition of test bacteria by *Glochidion tomentosum* extracts was lesser when compared to standard. Among bacteria, more inhibition was recorded in case of *P. aeruginosa* by extracts followed by *E. coli* and *S. aureus*. Standard antibiotic caused more inhibition of *S. aureus* followed by *P. aeruginosa* and *E. coli*. It appears that overall the microorganisms were found to be sensitive to ethanol and petroleum ether extracts of ***Glochidion tomentosum***. The reasons for this could be that the components active against microorganisms are most often obtained through solvent extraction.

Minimum Inhibitory Concentration (MIC) of ethanol and petroleum extract of *Glochidion tomentosum.* Among bacteria, *P. aeruginosa* was inhibited at low concentration (0.35 mg/mL) followed by *E. coli* (0.40 mg/mL) and *S. aureus* (0.50 mg/mL) by ethanol extract of *G. tomentosum*. Minimum inhibitory concentration of petroleum etherextract was found to be 0.30, 0.30 and 0.40 mg/mL for *E. coli*, *P. aeruginosa* and *S. aureus* respectively.

3.4. ANTHELMINTIC ACTIVITY OF SOLVENT EXTRACTS OF *GLOCHIDION TOMENTOSUM*:

The different concentrations of ethanol and petroleum ether extracts of *G. tomentosum* were evaluated for anthelmintic activity using adult Indian earthworm model. The ethanol and petroleum ether extracts have exhibited a dose-dependent inhibition of spontaneous motility (paralysis) and death of worms. Piperazine citrate (standard drug) at 1% concentration exhibited paralysis and death of worms at 78 and 104 minutes respectively. All the concentrations of ethanolic extracts of *G. tomentosum* showed greater potential than standard drug. In case of pet ether extract, the anthelmintic effects were comparable with that of 1% Piperazine citrate with higher doses namely 30mg/mL and higher. Overall, anthelmintic potential was higher in ethanol extract than petroleum ether extract.

Table-3.5: Anthelmintic activity of Ethanolic and petroleum ether extract of *Glochidion tomentosum.*

Treatment	Concentration	Time in minutes	
		Paralysis	Death
Normal saline	0.85%	-	-
DMSO	10%	-	-
Petroleum ether extract (mg/mL)	10	96	118
	20	88	106
	30	43	69
	40	20	31
Ethanol extract (mg/mL)	10	45	59
	20	20	35
	30	15	26
	40	11	15
Standard	1%	78	104

3.5. DPPH RADICAL SCAVENGING ASSAY OF SOLVENT EXTRACTS OF GLOCHIDION TOMENTOSUM

The result of antioxidant activity of different concentrations of standard (ascorbic acid) and petroleum ether and ethanol extracts of *Glochidion tomentosum*. The extracts have exhibited marked antioxidant activity by scavenging DPPH· (free radical) and converting into DPPHH. The extracts have shown concentration dependent radical scavenging activity. Ethanol extract showed 89.41 % of inhibition at 1 mg/mL concentration and petroleum ethershowed 79.05 % of inhibition at 1 mg/mL concentration. Furthermore petroleum ether and ethanol extract for radical scavenging activity was in the order of Ascorbic acid>ethanol extract>*petroleum ether.*

Table-4(a): Antioxidant activity of standard ascorbic acid by DPPH free radical scavenging assay

Concentration (µg/mL)	Radical scavenging activity (%) of ascorbic acid
20	11.71±0.46
40	22.22±.089
60	37.99±1.45
80	46.65±.0.70
100	62.16±.1.00

Table-4(b): Antioxidant activity of solvent extract of bark extract of Glochidion tomentosum by DPPH free radical scavenging assay

Concentration (mg/mL)	Radical scavenging activity (%)	
	Pet ether extract	*Ethanol extract*
25	20.55±0.78	14.50±0.50
50	42.95±2.38	27.50±.050
75	53.59±2.53	45.50±1.50
100	62.88±.1.35	65.49±.3.35
125	80.14±.1.09	84.51±.4.90

There are several methods available to assess antioxidant activity of compounds. An easy, rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 1, 1, diphenyl-2-picryl hydrazyl (DPPH) stable radical spectrophotometrically. In presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases. In this study, the scavenging activity of bark extract of *G. tomentosum* was found to be dose dependent i.e., higher the concentration, more was the scavenging activity. Though the DPPH radical scavenging abilities of the extracts were less than that of ascorbic acid, the study showed that the extracts have the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

4.11.3 FE^{+3} REDUCING POWER ASSAY

The result of reducing power of different concentrations of soxhlet extracts of selected G. tomentosum and tannic acid (standard) is presented in Table-4.20(a) and (b). In this study, the absorbance was found to increase with the dose of extract and standard which is suggestive of reducing power.

Table-4.20 (a): Antioxidant activity of tannic acid standards by Fe^{+3} reducing power assay

Concentration (µg/mL)	Radical scavenging activity (%) of ascorbic acid
20	0.16±0.01
40	0.33±.0.02
60	0.47±0.01
80	0.60±.0.01
100	0.76±.0.01

Table-4:20 (b) Antioxidant activity of soxhlet extract of G.tomentosum by Fe^{+3} reducing power assay

Concentration (mg/mL)	*Radical scavenging activity (%)*	
	Ethanol extract	*Peroleum ether*
25	0.27±0.01	0.19±0.01
50	0.32±0.01	0.20±.0.01
75	0.51±0.04	0.30±0.01
100	0.76±.0.02	0.63±.0.01
125	0.81±.0.01	0.70±.0.02

The presence of reductants such as antioxidant substances in the antioxidant samples causes the reduction of the Fe^{3+}/ ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Chung *et al., 2002)*. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Meir *et al.,* 1995). However, the antioxidant activity of putative antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging(Diplock, 1997).

4.11.4. ABTS SCAVENGING ACTIVITY OF BARK EXTRACT OF *G. TOMENTOSUM*:

The result of antioxidant activity of different concentrations of bark extracts of G. *tomentosum* and standard (ascorbic acid) is shown in Table-4.21 (a) and (b). The extracts have exhibited marked antioxidant activity by scavenging ABTS free radical and converting into ABTS. The extracts have shown dose dependent scavenging activity. Pet ether extract showed 82.70±1.560 % of inhibition at 60 µg/mL concentrations and Ethanol extract showed 69.31±1.350 % of inhibition at 60 µg/mL concentrations. Furthermore Pet ether extract and Ethanol extract radical scavenging activity was in the order of Ethanol extract>Pet ether extract compared with standard ascorbic acid.

Table-4.21 (a): ABTS scavenging activity of standard ascorbic acid

Concentration (µg/mL)	Radical scavenging activity (%) ascorbic acid
20	13.08±0.21
40	28.67±1.54
60	42.50±1.29
80	59.28±1.71
100	73.05±1.72

Table-4.21 (b): ABTS scavenging activity of bark extract of *G. tomentosum*

Concentration (mg/mL)	Radical scavenging activity (%)	
	Pet ether extract	*Ethanol extract*
25	18.70±1.19	14.44±0.84
50	40.10±1.56	29.40±1.89
75	57.25±1.01	41.56±2.32
100	75.10±1.77	52.24±1.10
125	82.70±1.56	69.31±1.35

CONCLUSION:

Considering the rich diversity of plants, it is expected that screening and scientific evaluation of endangered plant extracts for their antimicrobial activity may provide new antimicrobial substances. Since the study involved the different pathogens which are among most notable antibiotic resistant bacteria, and the extract showed potent antioxidant and anthelmintic activity could be useful as the medicine. Further experiments are to be conducted to isolate active principles from the extracts and their pharmaceutical potency need to be evaluated.

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