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Research Article

Comparative Study on Phytochemical and Antioxidant Properties of *Gmelina arborea* Roxb. From four Different Geographical Regions

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ABSTRACT

In the present study, leaf, bark, twig and root samples of *Gmelina arborea* (Family: Verbenaceae) are taken from four different geographical regions in and around Tamil Nadu. The samples were subjected to Soxhlet extraction with Methanol (MeOH) and n-Hexane. They were screened for the presence of phytochemicals and their respective concentrations were estimated and compared. Antioxidant activities of the selected samples were tested using 1-diphenyl-2-picrylhydrazyl (DPPH) Assay and Metal Chelation Assay. When compared to n-Hexane, All the samples of Methanol extract showed good concentrations of secondary metabolites, especially high phenol content (8.4- 4.4 mg/ml of MeOH extract). There were significant amounts of Alkaloids (2.6-0.1 mg/ml), Tannins (3- 0.1 mg/ml) and Saponins (8.2-0.2 mg/ml) though their concentration ranges were highly varying from sample to sample. The radical scavenging activity was stable and significant in all the samples from Area-3 and 4 with B3M the highest (71.51%). On a comparative scale, *G.arborea* samples from Area-3 and 4 (Farmers' plantations) showed a good amount of phytochemicals and antioxidant potential thus forming a good line of trees for selection and breeding.

Keywords: *Gmelina arborea*, Phytochemicals, Phenols, Antioxidant activity

INTRODUCTION

India is a country blessed with 12 mega biodiversity centres of the world with 16 agro-climatic zones with the claiming of 7000 species of medicinally potential herbs and trees. Such plants are considered to be pharmaceutically rich and are used in various systems of indigenous medical practices like Ayurveda, Siddha and Unani.

Gmelina arborea (Common name: Gambhar; Family: Verbenaceae) is one among the most highly treasured medicinal plant species which is being used in the treatment of fever, heart diseases, nerve disorders, a number of digestive and reproductive disorders[1]. This deciduous tree, indigenous to the tropical and subtropical region of Southeast Asia[2,3] has widespread medicinal values embedded in all of its parts. Roots and barks of this plant are used in preparing Ayurvedic tonics like *Dashmuladikwath*, *Kutajarista*, *Chyawanprasha* and *Bhrahat panchamool*[4]. Leaves and flowers are used in curing leprosy, vomiting, burning sensations and blood diseases[5].

Phytochemicals or the secondary metabolites are compounds present in plants that largely attributes to their therapeutic properties (antioxidant, anticancer etc.,) and play a crucial role in their biological activities[6]. Plant selection is an important aspect in farming and plantation of this tree to obtain high production of timber that is used for commercial purposes and Potential phytochemicals that can be used for medicinal purposes. The present study was aimed to determine the phytochemical constituents and antioxidant properties of *Gmelina arborea* tree samples isolated from four different geographical regions in and around Tamil Nadu so that it can facilitate the selection of plants for farming methods.

MATERIALS AND METHODS

Plant samples

Samples of leaves, barks, twigs and root of *Gmelina arborea* Roxb. were taken from four different agro-climatic regions as shown in Table 1. All the samples were identified and authorised under the supervision of Mr. A. Mayavel, Scientist, Institute of Forest Genetics and Tree Breeding, Coimbatore, India.

Extracts preparation

25g of the dried and powdered samples were subjected to Soxhlet extraction using Methanol and n-Hexane in repetitive cycles. After distillation, plant extracts were recovered from the solvent by subjecting to rotary evaporator and were stored in 4°C.

Phytochemical screening and estimation

All the extracts were screened for the presence of Phenols, Alkaloids, Flavonoids, Terpenoids, Tannins, Saponins and Sterols and the estimation for the same were performed with reference to standardised procedures[7].

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Table 1: Samples of *G.arborea* collected from different geographical regions.

Sample Area	Tree habitat	Place of collection
1	Natural forest	Siruvani, Coimbatore district, Tamil Nadu
2	Natural forest	Yercaud, Salem district, Tamil Nadu
3	Farmers plantation	Thuvarankurichi, Trichy district, Tamil Nadu
4	Farmers plantation	Panampalli, Kerala

In-vitro free radical scavenging assay

The methanol extracts of selected samples were validated for the presence of antioxidant activity by 1-diphenyl-2-picrylhydrazyl (DPPH) assay[8] and Metal chelating activity[9]. Different volumes (2 - 20µl) of plant extracts were made up to 40µl with DMSO and 2.96ml DPPH (0.1mM) solution was added. The reaction mixture was incubated in dark condition at room temperature for 20 min and the absorbance was read at 517nm. 3ml of DPPH was taken as control. Ascorbic acid (10mg/ml DMSO) was used as reference. The percentage of radical scavenging activity of the plant extracts was calculated using the following formula,

Radical Scavenging Activity (RSA) (%) = [(Abs control-Abs sample)/ Abs control]*100.

Metal chelating activity was measured as by adding 0.1 mM FeSO4 (0.2 mL) and 0.25 mM ferrozine (0.4 mL) subsequently into 0.2 mL of plant extract. After incubating at room temperature for 10 min, absorbance of the mixture was recorded at 562 nm Chelating activity was calculated using the following formula:

Metal chelating activity (%) = (Abs control – Abs sample)/ Abs control x 100

Where (in both the cases)

Abs control- absorbance of control reaction (without plant extract)

Abs sample- absorbance in the presence of a plant extract.

RESULTS

The preliminary phytochemical screening and estimation

The results of preliminary phytochemical scanning and the total amount of each phytochemical estimated (in mg/g) of *G.arborea* leaves, bark, root and twig extracts are shown in Table 2 and 3.

It was observed that the Methanol extracts of all the samples were found positive for phenols, flavonoids and saponins. The highest amount of phenol was estimated in T4M (8.509 mg/g). B4M showed high concentrations of all the secondary metabolites especially phenols (8.331 mg/g) and saponins (7.633 mg/g). Fair amounts of phenols ranging from 4.8 mg/g to 6.7 mg/g were estimated in the remaining samples. L1M contained the highest of saponins (8.262 mg/g). Only scarce amounts of flavonoids were estimated to be present in all of the test samples. The highest flavonoid concentrations were estimated in 0.017 mg/g in B4M, T3M, R3M and R4M.

Noticeable amounts of alkaloids ranging from 2.1-0.11 mg (Atropine equivalents per gram of sample) concentrations were observed. Higher levels were seen in twig extracts T2M (2.114 mg/g) and T3M (2.082 mg/g). Tannins and Terpenoids were found comparatively lower than the rest of the secondary metabolites. Though the highest quantity of tannin was seen in B4M (3.387 mg/g), the rest of the bark samples showed very less or no presence of it. T2M (3.097 mg/g), T4M (2.661 mg/g) and T3M (2.435 mg/g) showed bigger concentrations of tannins than the sample extracts of leaves and roots.

Most of the extracts were tested negative for the presence of terpenoids except the methanol extracts of all the samples from area 3 and 4. Higher concentrations were found in B4 (2.314 mg/g), T3 (2.367 mg/g) and R3 (2.375 mg/g). The n-Hexane extracts of all the samples were tested positive for the presence of sterols. Though higher concentrations were witnessed on bark and root samples, L3H (3.146 mg/g) showed the greatest amount of the compound.

The overall phytochemical constituent in n-Hexane extracts were found to be much below than those of the MeOH extracts. It can also be noted that the extracts from the sample area 3 and 4 showed the presence of all the phytochemicals with significant concentrations. Hence, all (leaf, bark, twig and root) of the methanol extracts from the farmers plantation i.e. sample areas 3 and 4 and four other methanol extracts of L1M, B1M, T2M and R1M from the natural forests were chosen for further studies.

Invitro free radical scavenging assay

The selected samples of *G.arborea* were tested for invitro radical scavenging potential using two different assays. The extracts showed distinct abilities to utilize the free radical in different assays as tabulated in Table 4. Only the extracts L1M, B1M, T2M and R1M showed high potential in metal chelating while the rest of the samples showed greater activity in the DPPH assay. B3M showed a highest potential to scavenge free radicals in DPPH assay. It can be also noted that all of the sample extracts that belong to farmers' plantations (Region 3&4) show considerable invitro antioxidant potential.

DISSCUSSION

Phytochemicals are non-nutritive compounds (secondary metabolites) that contribute to plants' immunity, flavour and colour[10]. In a general definition, they are the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack[11,12]. In recent times, it is evidently known that phytochemicals have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been compiled[13] and are classified on the basis of their respective protective function, physical characteristics and chemical characteristics[14].

Phytochemical analysis is crucial in a plant as it gives us a defined insight on its potential to act as an effective drug source. *G.arborea* had been proved to possess anti-diabetic, anti-microbial, anticancerous and various other pharmacological activities. Phytochemical screening of

Table 2: Results of phytochemical screening on *G.arborea* Plant sample extracts

Phyto-chemicals		Phenols	Alkaloids	Flavonoids	Terpenoids	Tannins	Saponins	Sterols
Samples		Leaf Samples						
L1	M	+	+	+	+	+	+	-
	H	-	+	-	-	-	-	+
L2	M	+	+	+	-	+	+	-
	H	-	-	-	-	-	-	+
L3	M	+	+	+	-	-	+	-
	H	-	-	-	+	+	-	+
L4	M	+	+	+	+	+	+	-
	H	-	+	-	+	-	-	+
		Bark Samples						
B1	M	+	+	+	-	+	+	-
	H	-	-	-	-	-	-	+
B2	M	+	+	+	-	-	+	-
	H	-	-	-	-	-	-	+
B3	M	+	+	+	+	-	+	-
	H	-	-	-	-	-	-	+
B4	M	+	+	+	+	+	+	-
	H	-	-	-	-	-	-	+
		Twig Samples						
T1	M	+	+	+	-	-	+	-
	H	-	-	-	-	-	-	+
T2	M	+	+	+	-	+	+	-
	H	-	+	-	-	-	-	+
T3	M	+	+	+	+	+	+	-
	H	-	-	-	-	-	-	+
T4	M	+	+	+	+	+	+	-
	H	-	+	-	-	-	-	+
		Root samples						
R1	M	+	+	+	-	+	+	-
	H	-	+	-	-	-	-	+
R3	M	+	+	+	+	+	+	-
	H	-	-	-	-	-	-	+
R4	M	+	+	+	+	+	+	-
	H	-	+	-	-	-	-	+

Note:
(+) for presence and (-) for absence
'M' stands for Methanol extract and 'H' for n-Hexane extracts.
The samples were referred to as L1, B1, T1, R1, L2, B2, T2, L3, B3, T3, R3, L4, B4, T4, R4- Leaf (L), bark(B), twig(T) and root(R) samples from area 1, 2, 3 and 4 respectively.

different parts of *G.arborea* showed the presence of phenols, alkaloids, flavonoids, terpenoids, tannins, saponins and sterols. Estimation results showed different amounts in each phytochemical concentration. Phenol and saponins amounts were exceptionally high. Phenolics show numerous properties beneficial to humans and its antioxidant potentials are important in determining their role as protective agents against free radical-mediated diseases[15]. High phenolic content in the samples T4M (8.509 mg/g) B4M (8.331 mg/g) can attribute to its high radical scavenging potential. Many saponins are well-known to be antimicrobial, to prevent mould growth, and to safe guard plants from insect attack. They are also considered a part of plants' defence systems, and so have been included in a large group of plant-protective molecules named phytoanticipins or phytoprotectants[16]. So, it is apparent that *G.arborea* can act as a good anti-microbial agent as suggested in some studies[17,18].

Other than Phenols and Saponins, phytochemicals like alkaloids and tannins were estimated in fair amounts in the samples *G. arborea*. In medicine, particularly in Asian (Japanese and Chinese) natural remedial methods, the tannin-containing plant extracts are used as astringents against diarrhoea; as diuretics against stomach and duodenal tumours[19], and as anti-inflammatory, antiseptic, antioxidant and haemostatic medications[20]. Alkaloids are noteworthy for the protecting and survival of plant species because they ensure their survival against micro-organisms (antibacterial and antifungal activities), insects and herbivores (feeding deterrents) and also against other plants by means of allopathically active chemicals[21]. Some alkaloids have stimulant property as caffeine and nicotine. Morphine is used as the analgesic and quinine as the

Table 3: Estimation of total concentrations of phytochemicals present in *G.arborea* leaf, bark, twig, and root extracts.

Phytochemicals (mg/g)		Phenols	Alkaloids	Flavonoids	Terpenoids	Tannins	Saponins	Sterols
Samples		Area-1 (Natural Forests)						
L1	M	5.653±0.310	0.900±0.052	0.007±0.001	0.324±0.011	1.839±0.087	8.262± 0.413	-
	n-H	0.111±0.007	0.183±0.061	-	-	-	0.921± 0.011	2.213± 0.105
B1	M	6.731±0.421	0.808±0.016	0.008±0.003	-	0.774±0.351	5.485±0.324	-
	n-H	0.045±0.002	0.128±0.007	-	-	-	0.249±0.061	1.437± 0.023
T1	M	0.555±0.033	0.584±0.052	0.002±0.001	-	-	1.013±0.097	-
	n-H	0.056±0.001	0.237±0.014	-	-	-	0.681±0.0427	0.670± 0.033
R1	M	4.852±0.251	1.580±0.100	0.007±0.001	-	2.371±0.094	7.790±0.334	-
	n-H	0.048±0.003	0.123±0.007	-	-	-	0.568±0.0234	2.975± 0.569
Samples		Area-2 (Natural Forests)						
L2	M	6.430±0.321	1.635±0.083	0.007±0.002	-	0.581±0.024	2.620±0.093	-
	n-H	0.091±0.004	0.219±0.010	-	-	-	0.415±0.015	0.203± 0.085
B2	M	4.424±0.221	0.644±0.032	0.005±0.001	-	-	1.694±0.087	-
	n-H	0.071±0.001	0.237±0.011	-	-	-	-	2.254± 0.099
T2	M	7.278±0.351	2.114±0.079	0.007±0.001	-	3.097±0.133	6.009±0.301	-
	n-H	0.114±0.007	0.498±0.024	-	-	0.290±0.014	-	0.381± 0.014
Samples		Area-3 (Farmers Plantation)						
L3	M	4.852±0.242	2.689±0.093	0.016±0.004	-	-	1.799±0.059	-
	n-H	0.015±0.003	0.105±0.009	0.003±0.001	0.523±0.025	0.661±0.027	3.118±0.102	3.146± 0.175
B3	M	7.047±0.352	0.224±0.071	0.011±0.002	0.736±0.035	-	1.039±0.076	-
	n-H	-	0.146±0.007	-	-	-	0.699±0.029	2.135± 0.184
T3	M	6.199±0.299	2.082±0.104	0.017±0.005	2.367±0.091	2.435±0.098	5.188±0.309	-
	n-H	0.020±0.001	0.110±0.002	-	-	-	1.205±0.084	0.979± 0.045
R3	M	6.440±0.262	0.840±0.042	0.017±0.003	2.375±0.102	1.016±0.078	3.965±0.146	-
	n-H	0.020±0.001	0.146±0.007	-	-	-	3.214±0.174	3.008± 0.154
Samples		Area-4 (Farmers Plantation)						
L4	M	6.404±0.296	0.324±0.082	0.016±0.008	1.088±0.296	1.403±0.521	4.035±0.195	-
	n-H	0.029±0.008	0.146±0.065	-	0.288±0.004	-	3.502±0.154	1.931± 0.571
B4	M	8.331±0.395	1.238±0.086	0.017±0.006	2.314±0.102	3.387±0.143	7.633±0.341	-
	n-H	0.044±0.011	0.187±0.043	-	-	-	1.886±0.0795	2.569± 0.096
T4	M	8.509±0.412	0.416±0.020	0.016±0.007	2.213±0.123	2.661±0.103	4.900±0.194	-
	n-H	0.036±0.003	0.137±0.058	-	-	-	1.293±0.0842	1.456± 0.286
R4	M	5.789±0.259	1.941±0.092	0.017±0.008	0.462±0.022	0.160±0.004	3.450±0.157	-
	n-H	0.004±0.001	0.196±0.059	-	-	-	1.057±0.0753	1.871± 0.421

antimalarial drug[22]. Terpenoids were found to be in tiny amounts in the present study. They were present only in samples from the Areas 3 and 4 in which, B4 (2.314 mg/g), T3 (2.367 mg/g) and R3 (2.375 mg/g) had high concentrations. It is appropriate to define that the presence of such phytochemicals makes *G.arborea* a potent plant for various pharmaceutical and medical purposes.

Despite of the wide range of variation in the results, antioxidant activity were found to be positive for both the DPPH assay and the Metal chelating assay on all the selected sample extracts. The antioxidant potential was evaluated using DPPH assay and metal chelating assay. The DPPH assay is reported to be a direct and trustworthy method for the determination of radical scavenging

Table 4: Invitro Free radical scavenging activity of sample extracts of *G.arborea*

Sample Area	Samples of *G.arborea* plant extracts (1mg/ml)	Free Radical Scavenging Activity (%)	
		DPPH Assay	Metal Chelating Assay
Area-1 (Natural Forests)	L1M	37.62	86.88
	B1M	19.05	87.2
	R1M	43.33	88.04
Area-2 (Natural forests)	T2M	2.86	87.3
Area-3 (Farmers Plantation)	L3M	46.18	11.02
	B3M	71.51	12.17
	T3M	61.08	12.91
	R3M	49.53	25.6
Area-4 (Farmers Plantations)	L4M	63.07	31.9
	B4M	57.91	0.73
	T4M	60.15	21.3
	R4M	52.14	34.73

activity in *G.arborea*, where the structure of electron donor (e.g. plant extract) is unknown[23]. Phenolics compounds such as flavonoids, phenolic acids and tannins that are commonly found in leaves, flowering tissues and woody parts like stems and barks[24] are considered to be the major provider to the antioxidant activity of medicinal plants and are primarily responsible for scavenging of reactive species (Reactive Oxygen Species or Reactive Nitrogen Species)[25]. The antioxidant activity of phenolic compounds is chiefly due to their redox properties, as the presence of hydrogen donors permit them to act as reducing agents. Thus, the more the total phenolic content in a test sample, the higher will be the number of free hydroxyl groups and so will be its free radical scavenging potential. In our study the maximum percentage inhibition was noticed in Bark extracts (B3M-71.51%) Many studies also attribute a high concentration of Flavonoids to a higher antioxidant potential of the plant[26,27,23] but in the present study worthy percentages of free radical scavenging activity were seen even in lower concentrations of flavonoids. Very few amounts of Flavonoids are seen in B4M, T3M, R3M and R4M (0.017 mg/g) and yet the same samples exhibited good range of free radical scavenging abilities in the DPPH Assay.

The presence of transition metal ions in a biological system could catalyse the Haber-Weiss and Fenton type reactions, resulting in generation of hydroxyl radicals (OH). However, these transition metal ions could form chelates with the antioxidants, which results in the suppression of OH generation and inhibition of peroxidation processes of biological molecules[28]. The leaf, bark, twig and root samples of *G.arborea* from natural forests used in this study showed the highest ability to form chelates while the rest of the samples from regions of Farmers plantation showed lower ability to form metal chelates and hence lower scavenging potential in the Metal chelating assay. However, the latter showed good potential to form free hydroxyl groups which might be the cause of their dominance in scavenging DPPH free radical.

CONCLUSION

The data obtained from the present study shows that *Gmelina arborea* is one of the trees with significant number and amounts of phytochemicals. Hence, the presence of rich secondary metabolite concentration can apparently make the plant a good source of nutrients and pharmacological supplements that can cure a variety of ailments. It can also be concluded that samples form the farmers' plantation region (Thuvarankurichi and Panampalli) are better in their phytochemical formulations and antioxidant potential. These trees can be chosen for farming and plantation through plant selection methods where they can serve as a good source of raw material for both commercial and medicinal purposes.

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