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PHYTOCHEMICAL AND ANTIOXIDANT POTENTIAL OF *GMELINA ARBOREA* ROXB. FROM DIFFERENT AGROCLIMATIC REGION OF TAMIL NADU AND KERALA

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ABSTRACT

Variation in the phytochemical and antioxidant activity of methanol and n-Hexane extract of leaf, barks, twig and root of *G. arborea* growing in different agroclimatic regions was evaluated. The methanol and n-Hexane extracts of plant parts were screened for the qualitative and quantitative analysis of phytochemicals and antioxidant activities such as DPPH and Metal chelating activity. The study revealed that the presence of carbohydrates, fatty acids, phenols, alkaloids, flavonoids, tannins, saponins, terpenoid and sterols in all the plant parts of *G. arborea* collected from different geographical regions. The plants from site III and site IV were found to have relatively high secondary metabolites within the antioxidant property than that of the samples from the site I & II. As a good resource of sampling material, *G. arborea* may have beneficial medicinal values.

KEYWORDS: *Gmelina arborea*, phytochemicals, antioxidant, agroclimatic, Geographical.

INTRODUCTION

The plant kingdom is a treasure house of potential drugs. Currently, there has been a rising awareness about the importance of medicinal plants. The plant drugs are easily obtainable, not as much of expensive, safe and original. The plants have been used for medicinal purposes during from thousands of years, and the clearest option today is to explore for therapeutically effective new drugs. According to World Health Organization (WHO), medicinal plants are the source to obtain the variety of drugs. About 80% of folks within developed countries are using traditional medicines and this has derived compounds from medicinal plants.[1] In India, huge amount of medicines had been used from plants or their extracts still many more plants warrant such expeditions *G. arborea* is one such a tree species need to be studied for the therapeutically effective drug.

Gmelina arborea Roxb. an important commercial timber tree belongs to the family *Lamiaceae*. It is a fast growing deciduous tree, occupying throughout India. It has grown varied environmental conditions. It is one of the most frequently used medicinal plants in the Ayurveda, an ancient Indian system of medicine. The roots, leaves, flowers, fruits and bark are used for various ailments in traditional medicine such as scorpion sting, snake-bites, diabetes, used for treating hallucinations, excess thirst, piles, abdominal pains, burning sensations, and fever. Moreover, crude extracts from this plant are reported to possess wound-healing properties, antidiarrheal activity, anti-oxidant activity, anti-diabetic activity, anti-inflammation and antiulcer activity.[2] Whereas, the phytoconstituents were also identified like flavonoids, steroids, alkaloids, glycosides, lignans, Luteolin, indole alkaloids, iridoid glycosides, hen triacontanol in different parts of *G. arborea*.[3]

Though the tree is growing in varied environment condition, agroclimatic and environmental conditions may influence the chemical composition and therapeutic properties of medicinal plant species. The reports have suggested that temperature, soil type and other environmental factors are responsible for the variations in the chemical constituents and antioxidant potentials of the plant from different geographical locations.[4] Confirmation of phytoconstituents and antioxidant activity of plants across varied agro-ecologies is necessary for the selection and formulation of the plant-based development of medicinal compounds. It is important to find the most suitable geographical area for the better growth and yield of medicinal plants. Therefore, the present study aimed to investigate the variation in phytochemical composition and antioxidant properties of extracts made from different parts of *G. arborea* growing in different agroclimatic zones of Tamil Nadu and Kerala.

MATERIALS AND METHODS

Plant material collection

500g of leaves, barks, twigs and roots of *G. arborea* were collected from four different agro-climatic regions namely site I (natural forest Siruvani, Coimbatore district, Tamil Nadu), site II (natural forest Yercaud, Salem District Tamil Nadu), site III (Plantation Thuvarankurichi, Trichy District, Tamil Nadu) and site IV (Plantation Panampalli, Kerala).

Plant sample extraction

Each sample was washed repeatedly with pure water to remove dirt and dust particles. They were shade dried. The dried plant materials were powdered and stored in an airtight container until extraction. About 25g of dried powders were put into the thimble (Whatmann No: 1 filter paper) for extraction by using Soxhlet apparatus. About 350 ml methanol and n-hexane were taken and the apparatus was run 48 hours at the 40°C temperature for the extraction. After distillation, plant extracts had been recovered from the solvent by subjecting to the rotary evaporator.

Qualitative analysis of phytochemical

The preliminary phytochemical screening was carried out following the method of Harborne.[5]

Estimation of phytoconstituents

Primary and secondary metabolites in different parts of *G. arborea* from different agroclimatic region were estimated by the following the methods collected for carbohydrates[6], estimation of fatty acids[7], estimation of total phenols[8], estimation of alkaloids [9], estimation of total flavonoids[10], estimation of Terpenoids[11], estimation of tannins[12], estimation of saponins[13] and estimation of steroids.[9]

Determination of antioxidant activities

The radical scavenging activity of extracts made from different parts of *G. arborea* was determined by using DPPH assay[14] and Metal chelating activity by Soler-Rivas et al.[15]

RESULTS

The main aim of the present investigation is to find out the variation in phytochemical constituent and antioxidant activity of *G. arborea* growing in the different agroclimatic region of Tamil Nadu and Kerala. The preliminary phytochemical screening of the leaf, bark, twig and root of *G. arborea* revealed the presence of carbohydrates, fatty acids, phenols, alkaloids, flavonoids, tannins, saponins, terpenoid and sterols (Table –1).

Quantitative analysis of phytochemicals

Carbohydrates

Site III and site IV have the highest concentration of carbohydrates compare with other sites. The highest amount of carbohydrates was found to be present in root of *G. arborea* collected from Panampalli, Kerala (10.62 mg/g) followed by Thuvarankurichi (climatic zone of Tamil Nadu) (8.08 mg/g), the bark extract from Panampalli 11.74 mg/g of consequently (**Table-2**) compared with Siruvani and Yercaud collections.

Fatty acids

The free fatty acid estimation revealed that the n-Hexane extracts have higher concentration compared with methanolic extracts. The root and bark collected from site III (5.67 mg/g and 4.85 mg/g) and site IV (4.61mg/g and 6.98 mg/g) showed the maximum concentration of free fatty acids (Table- 3).

Total phenol contents

The methanolic extracts of leaf, bark, twig and root showed the higher concentration of total phenol content in the entire study site. Twigs and bark had a high amount of phenols followed by leaf and root collected from site II (Yercaud) and site IV (Panampalli). The n-Hexane extracts have a lower amount of phenols than methanol extracts in the all the plant parts (Table- 4).

Alkaloids

Plant collected from site III and site II was the most suitable for the alkaloids, the high concentration of alkaloids were recorded in twig (Thuvarankurichi 2.08 mg/g and Yercaud 2.11 mg/g) and leaf (Thuvarankurichi 2.68 mg/g and Yercaud 1.63 mg/g) followed by root (Panampalli 1.94 mg/g, Yercaud 1.14 mg/g and Siruvani 1.58 mg/g) (Table- 5).

Total flavonoids and Terpenoids

The lower amount of flavonoids was observed in the methanolic extract of all plant parts of *G. arborea* collected from Thuvarankurichi 0.011- 0.017 mg/g and Yercaud 0.016-0.017 mg/g (Table- 6). The terpenoids were only present in notable amount in the methanolic extracts of *G. arborea* from site III (bark0.736 mg/g, twig- 2.367 mg/g and root- 2.375 mg/g) and site IV (leaf-1.088mg/g, bark- 2.314mg/g and twig- 2.213 mg/g) (Table- 7). Hence, *G. arborea* growing in Thuvarankurichi and Panampalli was from the suitable for the high amount of Terpenoids.

Tannins

The data for estimated amount of tannins in methanol and n-hexane extracts of *G. arborea* samples are given in Table-8. The methanolic extracts of the twigs from site II, site II and site IV contains significantly the highest amount of tannins (3.09, 2.43 and 2.66 mg/g) respectively. The highest tannin concentration (3.39 mg/g) was obtained in the bark extract of *G. arborea* collected from Panampalli (site IV).

Saponins

The data of quantified amount of saponins in methanol and n-hexane extracts of *G. arborea* samples were given in Table- 9. The estimation of saponins showed the highest amount in the methanolic leaf extracts from Siruvani collection (4.26 mg/g), bark from Panampalli

collection (3.63 mg/g) and root from Siruvani (4.79 mg/g) collection. The methanolic extracts of all the samples contained more saponins content than that of the n-hexane extracts.

Steroids

The estimation of sterols in methanol and n-hexane extracts of *G. arborea* samples were given in Table- 10. Sterols were found in the n-hexane extracts of the plant. The highest concentration was given by the root extracts followed by bark extracts. The root n-Hexane extract (Thuvarankurichi) has 3.008 mg/g of sterols and the rest of the samples of root and bark contained approximately similar properties of sterols.

Antioxidant activity

In the present study, the selected plant extracts were subjected to antioxidant activity by DPPH assay and Metal chelating activity and resulted in Table-11. Approximately 88% of free radicals were scavenged by the methanolic extracts of root, leaf and bark collected from the site I (Siruvani) and methanolic extract of twig from site II (Yercaud) in the metal chelating assay, where, it was found to be weak in scavenging the radicals in DPPH assay. The highest scavenging potential (71.51%) was recorded in methanolic extract of bark collected at site III (Thuvarankurichi) by DPPH assay (Fig-1). Antioxidant activities were found to be positive for both the DPPH assay and the Metal chelating assay on all the selected sample extracts.

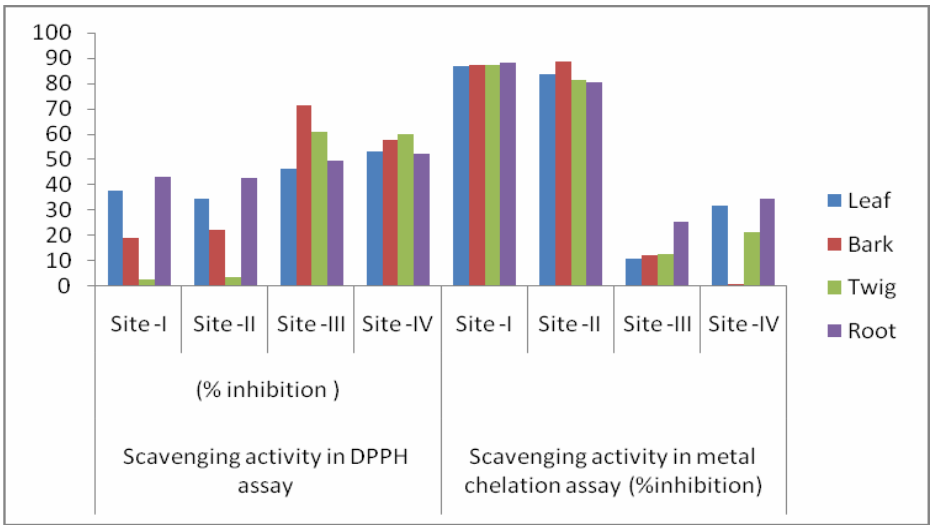

Figure 1: Antioxidant activity in methanolic extracts of various parts of *G. arborea* from different agroclimatic regions of Tamil Nadu and Kerala.

Table 1: Preliminary phytochemical screening of different parts of *Gmelina arborea* collected from different agroclimatic regions of Tamil Nadu and Kerala.

Geographical area	Parts	Extracts	Phytochemicals								
			Carbohydrates	Fatty acids	Phenols	Alkaloids	Flavonoids	Terpenoids	Tannins	Saponins	Sterols
Site I	Leaf	Methanol	+	-	+	+	+	+	+	+	-
		n- Hexane	+	+	-	+	-	-	-	-	+
	Bark	Methanol	+	-	+	+	+	-	+	+	-
		n- Hexane	+	+	-	-	-	-	-	-	+
	Twig	Methanol	+	-	+	+	+	-	-	+	-
		n- Hexane	+	+	-	-	-	-	-	-	+
	Root	Methanol	+	-	+	+	+	-	+	+	-
		n- Hexane	+	+	-	+	-	-	-	-	+
Site II	Leaf	Methanol	+	-	+	+	+	-	+	+	-
		n- Hexane	+	+	-	-	-	-	-	-	+
	Bark	Methanol	+	-	+	+	+	-	-	+	-
		n- Hexane	+	+	-	-	-	-	-	-	+
	Twig	Methanol	+	-	+	+	+	-	+	+	-
		n- Hexane	+	+	-	+	-	-	-	-	+
	Root	Methanol	+	-	+	+	+	+	+	+	-
		n- Hexane	+	+	-	-	-	-	-	-	+
Site III	Leaf	Methanol	+	-	+	+	+	-	-	+	-
		n- Hexane	+	+	-	-	-	+	+	-	+

	Bark	Methanol	+	-	+	+	+	+	-	+	-
		n- Hexane	+	+	-	-	-	-	-	-	+
	Twig	Methanol	+	-	+	+	+	+	+	+	-
		n- Hexane	+	+	-	-	-	-	-	-	+
	Root	Methanol	+	-	+	+	+	+	+	+	-
		n- Hexane	+	+	-	+	-	-	-	-	+
Site IV	Leaf	Methanol	+	-	+	+	+	+	+	+	-
		n- Hexane	+	+	-	+	-	+	-	-	+
	Bark	Methanol	+	-	+	+	+	+	+	+	-
		n- Hexane	+	+	-	-	-	-	-	-	+
	Twig	Methanol	+	-	+	+	+	+	+	+	-
		n- Hexane	+	+	-	+	-	-	-	-	+
	Root	Methanol	+	-	+	+	+	+	+	+	-
		n- Hexane	+	+	-	+	-	-	-	-	+

Note: + - present, - - absent

Table 2: Estimation of carbohydrates in various parts of *G. arborea* from different agroclimatic regions of Tamil Nadu and Kerala.

Parts	Extracts	Amount of carbohydrates (mg/g)			
		Site -I	Site -II	Site -III	Site -IV
Leaf	Methanol	5.45	5.49	9.71	10.78
	n- Hexane	0.83	5.23	0.32	0.58
Bark	Methanol	7.07	5.48	9.93	**11.74**
	n- Hexane	0	0	0	0
Twig	Methanol	4.71	5.48	**8.02**	12.64
	n- Hexane	0	0.61	0.04	0
Root	Methanol	5.50	6.28	**8.08**	**10.62**
	n- Hexane	0	0	0.32	0.02

Table 3: Estimation of fatty acids in various parts of *G. arborea* from different agroclimatic regions of Tamil Nadu and Kerala.

Parts	Extracts	Amount of fatty acids (mg/g)			
		Site -I	Site -II	Site -III	Site -IV
Leaf	Methanol	1.12	0.84	1.12	1.12
	n- Hexane	2.24	1.68	1.1 3	3.73
Bark	Methanol	0.84	0.84	0.56	2.81
	n- Hexane	2.24	1.12	4.85	4.61
Twig	Methanol	1.68	0.56	2.24	2.81
	n- Hexane	2.24	1.12	2.81	5.61
Root	methanol	0.84	0.81	1.12	1.12
	n- Hexane	2.24	1.67	5.67	6.98

Table 4: Estimation of total phenol contents in various parts of *G. arborea* from different agroclimatic regions of Tamil Nadu and Kerala.

Parts	Extracts	Amount of total phenols (mg/g)			
		Site -I	Site -II	Site -III	Site -IV
Leaf	Methanol	5.65	6.43	4.85	6.40
	n- Hexane	0.11	0.09	0.01	0.02
Bark	Methanol	6.73	4.42	7.04	8.33
	n- Hexane	0.04	0.07	0	0.04
Twig	Methanol	4.55	7.27	6.19	8.50
	n- Hexane	0.05	0.11	0.02	0.03
Root	methanol	4.85	4.36	6.44	5.78
	n- Hexane	0.04	0.03	0.02	0.004

Table 5: Estimation of alkaloids in various parts of *G. arborea* from different agroclimatic regions of Tamil Nadu and Kerala.

Parts	Extracts	Amount of alkaloids (mg/g)			
		Site -I	Site -II	Site -III	Site -IV
Leaf	Methanol	0.90	1.63	2.68	0.32
	n- Hexane	0.18	0.21	0.10	0.14
Bark	Methanol	0.80	0.64	0.22	1.33
	n- Hexane	0.12	0.23	0.14	0.18
Twig	Methanol	0.58	2.11	2.08	0.41
	n- Hexane	0.23	0.49	0.11	0.13
Root	methanol	1.58	1.15	0.84	1.94
	n- Hexane	0.12	0.01	0.15	0.20

Table 6: Estimation of total flavonoids in various parts of *G. arborea* from different agroclimatic regions of Tamil Nadu and Kerala.

Parts	Extracts	Amount of total flavonoids (mg/g)			
		Site -I	Site -II	Site -III	Site -IV
Leaf	Methanol	0.007	0.007	0.016	0.016
	n- Hexane	0	0	0.003	0.002
Bark	Methanol	0.008	0.005	0.011	0.017
	n- Hexane	0	0	0	0.001
Twig	Methanol	0.002	0.007	0.017	0.016
	n- Hexane	0	0	0.001	0.001
Root	methanol	0.007		0.017	0.017
	n- Hexane	0	0	0	0.001

Table 7: Estimation of terpenoids in various parts of *G. arborea* from different agroclimatic regions of Tamil Nadu and Kerala.

Parts	Extracts	Amount of terpenoids (mg/g)			
		Site -I	Site -II	Site -III	Site -IV
Leaf	Methanol	0.32	0	0	1.09
	n- Hexane	0	0	0.52	0.29
Bark	Methanol	0	0	0.73	2.31
	n- Hexane	0	0	0	0
Twig	Methanol	0	0	2.37	2.21
	n- Hexane	0	0	0	0
Root	methanol	0	0	2.37	0.46
	n- Hexane	0	0	0	0

Table 8: Estimation of tannins in various parts of *G. arborea* from different agroclimatic regions of Tamil Nadu and Kerala.

Parts	Extracts	Amount of tannins (mg/g)			
		Site -I	Site -II	Site -III	Site -IV
Leaf	Methanol	1.84	1.58	1.46	1.64
	n- Hexane	0	0	0.66	0
Bark	Methanol	0.77	1.24	1.03	3.39
	n- Hexane	0	0	0	0
Twig	Methanol	1.31	3.09	2.43	2.66
	n- Hexane	0	0.29	0	0
Root	methanol	2.37	1.87	1.01	1.16
	n- Hexane	0	0	0	0

Table 9: Estimation of saponins in various parts of *G. arborea* from different agroclimatic regions of Tamil Nadu and Kerala.

Parts	Extracts	Amount of saponins (mg/g)			
		Site -I	Site -II	Site -III	Site -IV
Leaf	Methanol	4.26	2.62	1.79	4.03
	n- Hexane	0.92	0.41	3.11	3.50
Bark	Methanol	2.48	1.69	1.04	3.63
	n- Hexane	0.25	0	0.69	1.88
Twig	Methanol	1.01	4.01	2.18	4.90
	n- Hexane	0.68	0	1.20	1.29
Root	methanol	4.79	4.43	3.96	3.45
	n- Hexane	0.57	0	3.21	1.05

Table 10: Estimation of steroids in various parts of *G. arborea* from different agroclimatic regions of Tamil Nadu and Kerala.

Parts	Extracts	Amount of steroids (mg/g)			
		Site -I	Site -II	Site -III	Site -IV
Leaf	Methanol	0	0	0	0
	n- Hexane	2.21	0.20	3.14	1.93
Bark	Methanol	0	0	0	0
	n- Hexane	1.45	2.25	2.13	2.57
Twig	Methanol	0	0	0	0
	n- Hexane	0.67	0.38	0.98	1.45
Root	methanol	0	0	0	0
	n- Hexane	2.97	2.58	3.008	1.87

Table 11: Antioxidant activity in methanolic extracts of various parts of *G. arborea* from different agroclimatic regions of Tamil Nadu and Kerala.

Parts	Scavenging activity in DPPH assay (% inhibition)				Scavenging activity in metal chelation assay (%inhibition)			
	Site -I	Site -II	Site -III	Site -IV	Site -I	Site -II	Site -III	Site -IV
Leaf	37.62	34.56	46.18	53.07	86.88	83.45	11.02	31.90
Bark	19.05	22.13	71.51	57.91	87.20	88.75	12.17	0.73
Twig	2.86	3.56	61.08	60.15	87.30	81.34	12.91	21.30
Root	43.33	42.65	49.53	52.14	88.04	80.44	25.60	34.73

DISCUSSION

Climate change is causing noticeable effects on the life cycle, distribution and phytochemical composition of the world's vegetation, including medicinal and aromatic plants. This climate changes influence in the plant architecture, flowering, fruiting, phytochemical composition and in situ competition with other species.[16] Hence, there is a need to understand the effect of geographical location on the phenology, nutrient, antioxidant activity and phytochemical composition of the medicinal plants. Hence, the present study analyzed variation on the phytochemicals and antioxidant activity of *Gmelina arborea* growing in the different agro-climatic regions of Tamil Nadu and Kerala.

The phytochemical analysis is commercially important for pharmaceutical industries to the production of new drugs to cure a variety of diseases. The secondary metabolites are significantly contributing to the biological activities and the plant materials contain numerous types of antioxidants with varied activities.[16] The present study revealed that the presence of carbohydrates, fatty acids, phenols, alkaloids, flavonoids, tannins, saponins, terpenoid and sterols in the leaf, bark, twig and root of the *Gmelina arborea* from different regions of Tamil Nadu and Kerala. Lawson et al.[17], Chellappan and Pemiah[18], Soni and Sosa[19] and Chothani and Patel[20] were also reported variation in phytochemical consentient of different parts of *G. arborea* collected from a different location.

The climatic condition, soil composition and other factors are influencing the synthesis of secondary metabolites. Consequently, the nutritional and phytochemical contents of the plant may vary from place to place.[21] This study also confirms the variations in the phytochemical amount. Consequently, Variation in agronomic conditions, season, climatic factors, water availability, light and CO2 are known to significantly affect phytochemicals consentient of the plants.[22] Secondary metabolites and antioxidants are generally known to increase under drought stress conditions and this is believed to be a response to an increase in oxidation damage. The plant pigments (Carotenoid) that can confer plants with resistance to the adverse effects of drought.[23] But in this investigation, the collections from

site III and site IV have higher secondary metabolites with high DPPH since these areas receiving high rainfall. Conflicting, the Metal chelating activity significantly high in sites I and II (natural forest) than a plantation, (sites III and IV).

Water being an integral part plays a vital role in the maintenance of plant life and one of the limiting factors to determining plant distribution and survival in a natural ecosystem. Stress treatment caused an increase in electrolyte leakage compared to control. Plant under height stress condition, where have a sharp increase in antioxidant enzyme activity.[24] The present investigations have higher antioxidant activity in both natural forests with natural stress. Medicinal plants contain some organic compounds which produce definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids[25,26] which supports the study. In the present study, variation in antioxidant activity was found both in the DPPH and the Metal chelating assay. The tree grows in the natural forest were found to be high antioxidant activity in Metal chelating assay than DPPH assay. This suggested that the samples collected from the natural forest may be considered as a suitable sample for therapeutic properties.

CONCLUSION

G. arborea is one of the trees that are found to have a number of medicinal and therapeutic properties. The present study has focused on a comparative view of the phytochemical concentration and antioxidant properties of various parts of the tree from different agroclimatic zones. From the four of the selected geographical regions, two of them were natural forests showed the high concentration of essential phytoconstituent with high antioxidant properties. As a rich source of phytochemicals, this plant would make an excellent source for drug development for various different diseases.

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