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INFLUENCE OF ALTITUDE ON THE PHYTOCONSTITUENTS AND ANTI-OXIDANT ACTIVITY OF THE LEAVES OF *TECTONA GRANDIS*

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Abstract:
The present study deals with the variations observed in quantity of phyto constituents in the leaves of Tectona grandis which were collected from different altitudes; Chikmagalur (3,400 feet)(TGC) and Mangalore (72 ft)(TGM) representing the higher altitude and lower altitude respectively. Phytochemical variations due to different regions were studied by quantification of total phenolic acids and total flavonoid content. Antioxidant activity of the two extract was carried out using DPPH .The results reveal that the amount of total phenolic acids and the flavonoids was more in case of the leaves collected from the higher altitude. The leaves collected from the higher altitude exhibited better antioxidant activity, which was attributed to the difference in the quantity of phytoconstituents in the two extracts. This study, to a certain extent gives proof that altitude is one of the environmental factors that can influence the quantity of phytochemicals.

Key words: *Tectona grandis, altitude, anti-oxidant, DPPH.*

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INTRODUCTION:

Tectona grandis belongs to the family verbenaceae. It is commonly referred to as teak and is reported to have various medicinal properties [1-3]. The phytoconstituents of the medicinal herbs have been reported to fluctuate with seasons and geographic regions [4] .Bioactive phytochemicals/secondary metabolites are prone to qualitative and quantitative variations depend on number of factors, some of them include harvesting period, stage of development, soil, rainfall and post-harvest methods [5]. Altitude is one such factor which has been reported to bring about changes in the quantity of phytoconstituents. Certain metabolites are only synthesized/ their contents significantly increase/decrease under specific environments [6].Furthermore,previous reports have demonstrated that herbs grown in different environments produce variation in secondary metabolites which in turn, results in differences in their healing properties [7]. Phenolic compounds like Phenolic acids, flavonoids and tannins are some of the important constituents in plant extracts [8].Quantification of the polyphenols and antioxidant activity of medicinal plants are some of the important tools for understanding importance of plants especially keeping in view their importance in health care [9].We have already reported the effect of plant stages on the phytoconstituents of *Tectona grandis* [10]. In the recent past the research on phytochemistry has been extensive; however, there is insufficient information regarding the effect of altitude on the phytochemical content. This study was designed with an aim to study the impact of altitude on the phytoconstituents of the plant under investigation. The leaves were collected from Chikmagalur and Mangalore in Karnataka state, India. Chikmagalur which is at an elevation of 1,090 meters (3,400 feet) above mean sea level .The district is between 12° 54′ 42′′ and 13° 53′ 53′′ north latitude and between 75° 04′ 46′′ and 76° 21′ 50′′ east longitude, in the Mallenadu region of Karnataka [11], while Mangalore is located at 12.87°N 74.88°E in the Dakshina Kannada district of Karnataka. It has an average elevation of 22 meters (72 ft) above mean sea level [1].

MATERIALS AND METHODS:

The leaves of *Tectona grandis* were collected from the rural areas of Chikmagalur and Mangalore in the month of October and shade dried. The dried leaves were pulverized to get a coarse powder and preserved in air tight container until further use. The leaves were extracted using methanol in Soxhlet apparatus, vacuum dried and stored.

Physicochemical parameters like Moisture content, Alcohol soluble extractive, Water soluble Extractive, Ash value, Acid insoluble was also determined and compared as per standard methods [13].

The extracts were subjected to qualitative analysis for the phytoconstituents like alkaloids, carbohydrates, glycosides, steroids, tannins and flavonoids as per the standard procedures. The total phenolic acid content and flavonoid content was determined by Folin ciocalteu method and Aluminum chloride colorimetric method using standard procedures. The phenolic content was obtained from the standard curve of gallic acid and was expressed as gallic acid equivalent. The absorbance was recorded at 750 nm in the UV spectrometer. While total flavonoid content was calculated as quercitin equivalent. Quercitin was used as the standard and the absorbance was measured at 415 nm.

The anti-oxidant activity of the extracts was evaluated using 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH). The stock solution of the extract was prepared in methanol. Further the working solutions of the extracts were prepared from the stock solution using suitable dilutions. The anti-oxidant activities of the extracts were determined. DPPH was prepared as 0.002 % solution in methanol and mixed with 1ml of both the standard and the samples. The prepared solutions were kept in the dark for ½ an hour and the absorbance was measured at 517 nm [14].

RESULTS AND DISCUSSION:

Environmental factors strongly affect the metabolism and accumulation of phytoconstituents. The quality and quantity of active constituents are regulated by most plants depending on the environmental conditions. The Folin ciocalteu method which is one of the important methods for the quantification of plant phenolics was used to quantify the amount of phenolic acid present in the extracts. The total flavonoid content for both the extracts was evaluated using aluminum chloride colorimetric method and was calculated as quercitin equivalent. In this study, significant differences were observed in the quantity of active constituents in the leaves of *Tectona grandis* growing in regions of two different altitudes. The amount of phenolic acids and flavonoids present in the plant collected from higher altitude was more as shown in table 2. This is in concurrence with reported literature [15-17]. This increase in the phenolic content with increase in altitude may be attributed as a response of plants to increased UV-B radiation and decreased temperatures.

Table 1: Moisture, Alcohol soluble,water soluble extractive values

Extract	Moisture content	Alcohol soluble extractive	Water soluble extractive	Ash value	Acid insoluble ash
TG(C)	11.2	4.9	6.8	3.6	0.63
TG(M)	8.5	3.4	5.3	3.4	0.61

The value of moisture content, alcohol soluble extractive and water soluble extractive were high in TGC, while there was no significant difference in the ash value and acid insoluble ash value of the two specimens.

Table 2: Total phenolic acid and flavonoids in the methanolic and aqueous extracts

Plant	Extract	Total phenolics µgm	Total flavonoid µgm
TG(C)	Methanolic	210	194
	Aqueous	174	152
TG(M)	Methanolic	168	156
	Aqueous	92	132

Anti-oxidant activity of TG(C) and TG (M):

The anti-oxidant activity was evaluated by the DPPH method, which is an easy, rapid and sensitive method to evaluate the anti-oxidant properties of extracts. Phenolic compounds play an important role in anti-oxidant activity. They may exert their activity by scavenging some reactive species, suppressing lipid peroxidation recycling, may bind pro-oxidant metals, and some may increase the activity of antioxidant enzymes [18]. The results of the anti-oxidant activity revealed that TG(C) exhibited better activity when compared to the TG(M) .This was attributed to the presence of phenolic compounds like flavonoids and phenolic acids ,the amount of which was more in TG(C).

Table 3: Anti-oxidant activity of the extracts:

µg/ml	TG(M)		TG(C)	
	abs	% inh	abs	% inh
10	0.080	75.60	0.039	88.10
20	0.074	77.43	0.036	89.02
40	0.070	78.65	0.034	89.63
80	0.068	79.26	0.030	90.85
100	0.061	81.40	0.028	91.46
120	0.059	82.01	0.026	92.07
140	0.054	83.53	0.023	92.98
180	0.049	85.06	0.021	93.59
200	0.043	86.89	0.019	94.20
250	0.038	88.41	0.018	94.51

CONCLUSION:

The present results indicate a chemical variation among the phenolic acid and flavonoids present in the leaves of *Tectona grandis* collected from different altitudes. The chemical instability of the plants is an important factor that should be taken into account while cultivating the plant material and application of proper biotechnological approach would help to exploit the plant on commercial basis such that selection of cultivation area for obtaining better yield of the active phytoconstituents becomes easier.

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