ANTHOCYANIN CONTENT OF TEAK AND ITS POTENTIAL THERAPEUTIC TRAITS: SOME OBSERVATIONS

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ABSTRACT

Currently, the natural herbal products are used either as nutrient or as nutraceutical to mitigate many of the life style disorders. \textit{Tectona grandis} Linn. (Lamiaceae), the timber tree which is widely distributed throughout the tropical belts of the world. Its leaves, barks, seeds and flowers have been used in the local traditional system as therapeutic to treat many diseases. Usage of teak wood in Ayurveda or Unani medical system as acrid, cooling agent and also for the treatment of headache, constipation, biliousness, burning sensation and pain, liver-related disorders, wormicide, cough, bactericidal, fungicidal, piles, leucoderma and dysentery infections. Many research works proved that the different parts of teak plant possesses antioxidant, antidiabetic, antimicrobial, hepatoprotective, antiulcer, antifertility, anti-inflammatory and antigenotoxic features which helps in the treatment of many disorders. The coloured extract of teak has been used in many crude drug mix and soft drinks. Many studies were carried in animal model to evaluate young leaf extracts as antidiabetic and antioxidant. This study aims to quantify anthocyanin content from the different parts of teak and its plausible medicinal applications.

KEYWORDS: \textit{Tectona grandis}, Verbenaceae, Stem Bark, Leaves, Toxicity, Therapeutic Values, Anthocyanin.

INTRODUCTION

The drug industries that utilize nearly about 30 to 35 herbal plants as medicine or documented and certified traditional medicines that are with written treatise in Ayurveda, Siddha, Unani, Amchi and Tibetan systems of medicine that use about 1,200 to 2,000 herbal species or as local health traditions that are based on traditions practiced by venders, folk...
healers, vaidyas, and tribal communities who considered more than 8,000 species herbal plants for primary health needs.[1]

Anthocyanins are coloured water-soluble molecules of polyphenolic class. The coloured pigments were in glycosylated forms. Anthocyanins results into variable colours such as red, purple, and blue in the fruits, leaves and vegetables. Berries, currants, grapes, and certain tropical fruits have rich anthocyanins levels. Red to purplish blue-coloured vegetables, grains, roots, and tubers that contains relatively rich amount of anthocyanins. Among the different anthocyanin pigments, cyanidin-3-glucoside represents the major fraction of anthocyanin distributed in most of the plant species. The colored anthocyanin was traditionally employed as a natural food colourant from time immemorial. The nature of colour and its stability are influenced by pH, light, temperature, and structural components including the sugar moieties. In acidic pH, anthocyanins forms red colour but changes in to blue when the pH enhances. Chromatography techniques have been massively employed in the extraction, separation, and estimation of anthocyanins. Besides the role of anthocyanidins and anthocyanins as natural colouring agents, they are potential pharmaceutical components that yield diverse beneficial health impacts. Researchers, like cell culture studies, animal models, and human clinical trials, revealed that anthocyanidins and anthocyanins possess antioxidative, microbicidal activities, improve eye and neurons health, and defense against different non-communicable diseases. These results substantiate the health impacts of anthocyanidins and anthocyanins, which may be due to their antioxidant potentials. Different mode and pathways are reported in the protective roles, including free-radical scavenging, cyclooxygenase regulating, mitogen-activated protein kinase pathway, and inflammatory cytokines signaling pathways. Therefore, this study focuses the quantification of anthocyanins from different parts of teak plant and their possible roles as natural food colourants and their nutraceutical properties for health.

MATERIALS AND METHODS

Plant material: The healthy fresh leaves of Tectona grandis were collected from wild teak plantations of Nilambur hills, Kerala, identified by referring floras and confirmed by authenticating with the herbaria of Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), Palode, Thiruvananthapuram.

Isolation and quantification of anthocyanin: 1g leaf sample was homogenized in 3 mL methanol containing 1% HCl and vortexed for 30 sec and kept in water bath at 60° C for 20
min. Then the sample was centrifuged at 10000 rpm for 10 min. The supernatant was made up to a known volume and kept at 0°C for further analysis.

1 mL of the extract was prepared for two dilutions of sample, one adjusted with KCl buffer (pH 1.0) and the other with sodium acetate buffer (pH 4.5). These dilutions were equilibrated for 15 min. The absorbance of each dilutions were read at 510 and 700 nm against distilled water as blank.\textsuperscript{[2]}

**Statistical analysis:** The data obtained was mean of six independent experimentals ± SD and was statistically subjected to analysis of variance (ANOVA). The significance was recorded at $p < 0.05$ using SPSS package version 15.0.

**RESULTS AND DISCUSSION**

As an initial part of the study, the anthocyanin content from different parts of teak was quantified. The plant tissue was homogenized using 1% (v/v) HCl in methanol and centrifuged at 10,000 rpm for 10 min at room temperature. The supernatant of known volume was used for the quantification of anthocyanin. High amount of anthocyanin (6.4 mg/g) was quantified from the tender leaves of teak followed by mature leaf, bark, seeds and flowers (Table 1).

**Table 1: Quantification of anthocyanin from different parts of *Tectona grandis*.**

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>Young leaf</th>
<th>Mature leaf</th>
<th>Seeds</th>
<th>Bark</th>
<th>Flower</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monomeric anthocyanin content (mg/g)</td>
<td>6.4</td>
<td>3.25</td>
<td>1.32</td>
<td>2.02</td>
<td>0.84</td>
</tr>
</tbody>
</table>

The pH differential method and HPLC are commonly used for quantifying anthocyanin from tissue samples. Lao and Giusti compared the efficiency of four different techniques for the quantification of anthocyanin.\textsuperscript{[3]} Among the four methods evaluated, total anthocyanin method yielded highest anthocyanin value, followed by pH differential and other methods with intact pigments. Meanwhile, the HPLC with hydrolyzed pigments yielded lowest anthocyanin content. Eduardo et al. assessed the total anthocyanin content from 10 cultivars of muscadine grapes using pH differential method and the value ranged from 1.1 to 95.3 mg/100g FW.\textsuperscript{[4]} Gouvea et al. quantified anthocyanin of fruits from *Neomitrantes obscura*, an endemic species from Brazil and compared with different chromatographic techniques.\textsuperscript{[5]} Normally tomato fruits produce small amount of anthocyanin, but the transgenic tomato yielded significant amount of anthocyanin due to the expression of two transcription factors.
namely Del and Rose.\textsuperscript{[6]} Zhang \textit{et al.} made a comparison on the anthocyanin content from three different coloured onion and found that red coloured onion yielded highest amount of anthocyanin i.e., 29.99 mg/100 g FW followed by yellow and white coloured onion (9.64 and 0.75 mg/100g FW).\textsuperscript{[7]} Zoratti \textit{et al.} evaluated the effect of monochromatic light for the enhanced production of anthocyanin during the fruit development of bilberry.\textsuperscript{[8]} The plants illuminated with monochromatic lights such as blue, red and far red yielded significant amount of anthocyanin when compared with white light or plants kept under dark. The increased level of anthocyanin may be due to the accumulation of delphinidin glycosides.

Sipahlı \textit{et al.} analyzed the stability of crude anthocyanin extracted from calyces of \textit{Hibiscus sabdariffa} in terms of heat, light and pH.\textsuperscript{[9]} They found that approximately 87\% of pigments were retained when heated at 50\(^0\)C and reduced to 61\% at 80\(^0\)C. They also observed that the anthocyanin stability degraded slowly at acidic pH and samples incubated under dark. Yin \textit{et al.} made a comparison on the anthocyanin content from ten pigmented potato cultivars native to China and found that significant amount of total anthocyanin content (TAC) was obtained from peels of the potato when compared with flesh and whole tuber irrespective of cultivars (TAC range from 59-293 mg CGE/100g FW in the potato peel).\textsuperscript{[10]}

Anthocyanin is one of the major bioactive molecules as nutraceutical and local medicine. It has been employed as a biopharmaceutical, appetizer, choleretic molecule, and for the treatment of different diseases. These coloured molecules are potent nutrient or pharmaceutical agents. As a nutraceutical, its bioavailability is the major factor for maintaining optimal health and for the prevention of disorders.

The major health and therapeutic roles of anthocyanin was contributed by its antioxidative potentials. Miguel reviewed anthocyanin chalcones and quinoidal bases with a double bond conjugated to the keto group were efficient antioxidant molecules in inhibiting free radicals.\textsuperscript{[11]} Also, Vilas Boas \textit{et al.} reported that the glycosylated B-ring structure of the molecules contributes to the high antioxidant potentiality, where ortho-hydroxylation and methoxylation substantially enhance the antioxidant power.\textsuperscript{[12]} Murukan and Murugan proved the antioxidant potentials of teak anthocyanin using various assays.\textsuperscript{[13]} Generally, anthocyanidin possess a higher ORAC value than anthocyanin. The plausible reason is anthocyanin aglycone is highly unstable and reactive. Further, the addition of an extra sugar at C-3 position in the heterocyclic C-ring, has reduced antioxidant activity than the anthocyanidin with a single sugar moiety. Acylation of anthocyanin with phenolic acid has a
sound increase in antioxidant activity. Diacylation of the anthocyanin enhance the antioxidant activity but 5-glycosylation leads to a decrease in the activity.\[14\]

Angiogenesis is a physiological process in humans was regulated by the endothelial cells and supported by anthocyanin. Derailment in the angiogenesis may leads to many human disorders, including CVDs, tumors, and diabetic issues like retinopathy and nephropathy disorders. Healthy angiogenesis depends on the intricate balance between angiogenic such as VEGF, FGF2-fibroblast growth factor, TGF-β-transforming growth factor, and angiopoietin and antiangiogenic factors - angiostatin, endostatin, and thrombospondins.\[15\]

Epidemiological research works documented the correlation between anthocyanin and cardiovascular diseases. In vitro anti-thrombotic effect was supported by anthocyanin containing maize seed in the diet fed rats which were less susceptible to ischemia-reperfusion injury and reduction of infarct size with increased myocardial antioxidant enzyme.\[16\] Alvarez-Suarez et al. recorded that anthocyanin rich extracts of fruits possess vasorelaxation properties by regulating nitric oxide which is a potent vasodilator agent.\[17\]

Anthocyanins are anticancer agents and antiangiogenesis regulators revealed by in vitro and cell culture and animal model studies. Antiangiogenesis regulates the formation of fresh blood vessels that supply oxygen to the tumor cells.\[18,19\] Similarly, antidiabetic effect of anthocyanins of Cornus fruits have been documented in traditional Chinese prescription medicines against diabetes. Primary bioactive components reported in Cornus fruits are the glycosides of cyanidin, delphinidin, and pelargonidin.\[20\] Jayaprakasam et al. reported that cyanidin- and delphinidin-3-glucoside strongly regulates insulin secretion from rodent pancreatic β-cells under in vitro studies.\[21\]

Anthocyanin pigments are important nutraceuticals in maintaining vision. For example berries are known for eyes and associated with night vision. Oral administration of bilberry anthocyanin extract to mice has been shown to prevent impairment of photoreceptor cell function during retinal inflammation.\[22\] Similarly, 132 patients with glaucoma were supplemented with 60 mg anthocyanin capsules of bilberry daily and have improved visual function, based on the Humphrey visual field test and minimal angle of resolution best-corrected visual acuity analysis.\[23\]
Anthocyanin possess anti-obesity properties for example, Tsuda et al. reported that obese mice fed a diet rich in cyanidin-3-glucoside from purple corn for 3 months have reduced body weight, followed by decreases in white and brown adipose tissue weights.\textsuperscript{[24]} Purple corn diet regulates hyperglycemia, hyperinsulinemia, hyperleptinemia through tumor necrosis factor (TNF-\(\alpha\)) mRNA level. The purple corn also suppresses mRNA levels of enzymes involved in lipid synthesis and lowered sterol regulatory protein-1 mRNA level in the white adipose tissue. These down regulations regulate triacylglycerol accumulation in white adipose tissue.

Anthocyanins also possess microbicidal activity against many pathogens, especially in food-borne pathogens.\textsuperscript{[25]} The mode of action may be through cell damage by cell wall, membrane disruption, and unbalancing intercellular matrix. Further, anthocyanins function as neuroprotective agent that prevents the nervous system from secondary injuries. \textit{In vitro} and \textit{in vivo} studies based on animal models proved this effectively.\textsuperscript{[26]}

Significant differences for leaf and other parts in terms of anthocyanin content was noticed among the teak tested. Anthocyanin indexes remained stable for the majority of the species; however certain species showed variations in connection with season. Anthocyanins provide chemical defenses against pests and may help to protect plants during excessively winter.

Colour is the most unique quality attributes affecting the consumer's choice of food since it gives indirectly the food quality.\textsuperscript{[27]} Wills et al. documented that the pH value of Arab Roselle was 2.62.\textsuperscript{[28]} This increase in pH throughout maturation was due to metabolic events in the fruits that resulted in the decrease of organic acids a source of respiratory energy in cells. So, the extracting solution should be acidic to maintain the flavyluum cation form, which is red and stable in acidic medium, but not too acidic to cause partial hydrolysis of the acyl moieties in acylated anthocyanins.\textsuperscript{[29]}

**CONCLUSION**

Anthocyanins are coloured molecules dominating in flowers, fruits and seeds of plants that possess many health benefits. These pigments seem to red in acidic condition and blue hue in alkaline pH. Acylated and co-pigmented anthocyanidins have heat stability, thus maintain the structure under different pH conditions. In teak, anthocyanin content was significant in the young leaves as compared to mature. Anthocyanin displays many biological properties.
Future studies are warranted to analyze the biological potentialities of these unique plant pigments in the teak plants.

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REFERENCES


