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Phytochemical Screening, Assessment of Mineral Content and Total Flavonoid Content of Stem Bark of *Dalbergia Lanceolaria* L.

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ABSTRACT

In this research work, the *Dalbergia Lanceolaria* L., Myanmar name Thit pagan was selected to qualify and quantify the flavonoids present in it. The stem bark of Thit pagan was collected from Minbu Township, Magway Region, Myanmar. Firstly, the preliminary phytochemical test of this selected plant was carried out which gave positive for glycoside, flavonoid, polyphenol phenolic, sugar, saponin, tannin, terpene, alkaloid, and lipophenol test. Furthermore, the mineral contents of selected sample were measured by EDXRF method. Moreover, the total flavonoids of selected sample were extracted with 95% EtOH. This extract was checked for qualitative test of flavonoids. It responds positive for Ferric Chloride test, Shinoda's test and Lead Acetate test respectively. In addition, total flavonoid content of *Dalbergia Lanceolaria* L. was evaluated by the aluminum chloride ($AlCl_3$) method using UV/Visible spectrophotometer (UV-1800, SHIMADZU, UV spectrophotometer) at 510 nm. The total flavonoid content of this selected sample was determined as 41.17 ± 0.11 mg quercetin equivalent (QE) per gram dry weight.

KEYWORDS: *Dalbergia Lanceolaria L., flavonoids, phytochemical test, EDXRF method, UV/Visible spectrophotometer, quercetin.*

1. INTRODUCTION

Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times. Plants synthesize hundreds of chemical compounds for functions including defense against insects, fungi, diseases, and herbivorous mammals. Numerous photochemical with potential or established biological activity have been identified.

However, since a single plant contains widely diverse photochemical, the effects of using a whole plant as medicine are uncertain. Further, the photochemical content and pharmacological actions, if any, of many plants having medicinal potential remain unassisted by rigorous scientific research to define efficacy and safety [1].

Plant medicines are in wide use around the world [2]. In most of the developing world, especially in rural areas, local traditional medicine, including herbalist, is the only source of health care for people, while in the developed world, alternative medicine including use of dietary supplements is marketed aggressively using the claims of traditional medicine. As of 2015, most products made from medicinal plants had not been tested for their safety and efficacy, and products that were marketed in developed economies and provided in the undeveloped world by traditional healers were of uneven quality, sometimes containing dangerous contaminants [3].

In recent years, many researchers have focused on medicinal plants derived from natural products due to their wide range of pharmacological significance [4]. Moreover, natural resources of vegetable origin represent an important source of drugs in the process of developing new pharmacologically active compounds [5]. The World Health Organization established that, in many developing countries, traditional medicine plays an important role in meeting the primary health care needs of the population, and highlights specific types of this medicine [6].

Dalbergia lanceolaria L. (Fabaceae), commonly known as dandusi, is a shade tree grown in rural areas of India. The wood of the plant is used for tool handles and agricultural implements and is suitable for carving, boarding, rafters, packing cases, and general construction purposes. Decoction of the bark is used for dyspepsia, and the seed oil is used for rheumatism [7].

Dalbergia lanceolaria L. is a species of tree in the subfamily Faboideae and tribe Dalbergieae [8]. It is a medium-sized tree growing to 20 m tall and is native to: India, Sri Lanka, Nepal, Myanmar and Indo-China [9, 10].

The bark of the tree is traditionally used as an analgesic and anti-diarrhoeal [11]. The apiose isoflavone compound lanceolarin is found in its root bark [12].

Antioxidants are the agents that can interfere with the oxidation process by various mechanisms, such as, reacting with free radicals, chelating free catalytic metals, and acting as oxygen scavengers [13, 14]. There is an increasing interest in natural antioxidants, namely phenols, present in medicinal and dietary plants, that might help prevent oxidative damage [15, 16]. The administration of an antioxidant source comprising of multiple components could offer protection against cancer and combat oxidative stress – induced physiological malfunctions [17].

Presently, the use of synthetic antioxidants has been criticized. It is usually implied that regular consumption of natural antioxidants from vegetables, fruit, tea, and herbs may contribute to a shift in balance toward an ample antioxidant status. The interest in natural antioxidants, especially phytochemicals has greatly increased in recent years [18]. Many phytochemicals including phenolics, flavonoids, tannins, proanthocyanidins, and various herbal extracts have been reported as antioxidants [19, 20].

The aim of this study was to investigate the phytochemical constituents, mineral content and total flavonoid content of *Dalbergia lanceolaria* L.

1.1 Botanical description

Family Name - Fabaceae
Botanical Name - *Dalbergia Lanceolaria* L.
Myanmar Name - Thit pagan
Genus - Dalbergia
Medicinal uses - dyspepsia, anti-inflammatory, rheumatism, antiarthritic

Figure 1: Plant, flower and stem bark of *Dalbergia Lanceolaria* L.

2. MATERIALS AND METHODS

2.1. Sample collection

The stem barks of *Dalbergia Lanceolaria* L. were collected from Minbu Township, Magway Region, Myanmar. It was cut into small pieces and dried in the air for about four weeks. It was stored in a well-stoppered bottle and used throughout the experiment.

2.2. Preliminary Phytochemical Test of *Dalbergia Lanceolaria* L.

The phytochemical screening of stem bark of *Dalbergia Lanceolaria* L. was done.

2.3. Determination of Mineral Content from the Stem Bark of *Dalbergia Lanceolaria* L.

Mineral contents of the stem bark of *Dalbergia Lanceolaria* L. were measured at the Department of Physic, University of Mandalay, Myanmar by applying EDXRF (Energy Dispersive X-ray Fluorescence Spectroscopy). The results were tabulated in Table 2.

2.4. Extraction Procedure

2 g of dried plant sample was ground in a mortar and pestle. It was extracted with 30 mL of 95 % EtOH. The mixture was centrifuged at 5000 rpm for 30 minutes. The supernatant was decanted to a small beaker. The extraction procedure was repeated for two times. The supernatant was poured to the same container. 54 mL of extraction solution was obtained. This extract contains the flavonoids. 1 mL of this extraction was made 100 mL of solution with distilled water.

2.5. Qualitative Test for Flavonoids

Ferric Chloride Test:

A few drops of neutral ferric chloride solution were added to 1 mL of extract solution. Formation of blackish red color indicates the presence of flavonoids.

Shinoda's Test:

To 1 mL of extract solution, a small piece of magnesium ribbon or magnesium foil was added and a few drops of concentrated HCl were added. Change in pink red colour shows the presence of flavonoids.

Lead- acetate Test:

To 1 mL of extract solution, a few drops of aqueous basic lead acetate solution were added. Formation of precipitate indicates the presence of flavonoids.

2.6. Quantitative Determination of Total Flavonoid Content

2.6.1. Principle

The basic principle of Aluminium chloride colorimetric method is that aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition it also forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B- ring of flavonoids. Quercetin is reported to be suitable for building the calibration curve. Therefore standard quercetin solutions of various concentrations were used to build up the calibration curve [21-24].

2.6.2. Preparation and Determination of Standard Quercetin

10 mg of the standard quercetin was taken in a test tube. 100 mL of distilled water was added to the standard compound. The stock solution was obtained. It was diluted with distilled water in various ratios to obtained five ranges of concentration, such as 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, and 50 µg/mL respectively. Then, 5.0 mL of solution was prepared for each concentration. A 0.5 mL of quercetin at different concentrations was put in test tubes. At the onset of the experiment, 150 µL of 5% $NaNO_2$ was added to the test tube. After 5 min, 150 µL of 10% $AlCl_3$ was added. After further 6 min, 1 mL of 1M NaOH was added to the mixture. The solution was diluted to a final volume of 5 mL with water and mixed. After each standard solution was mixed with distilled water, the absorbances of the solutions were

measured with a UV/Visible spectrophotometer at 510 nm with respect to the blank solution [21-24].

2.6.3. Determination of Total Flavonoid Content of *Dalbergia Lanceolaria* L.

The total flavonoid content of extract of *Dalbergia Lanceolaria* L. was measured by aluminium chloride ($AlCl_3$) according to a colorimetric assay using quercetin as a standard. Firstly, 0.5 mL of extract solution were taken in each test tube. 150 µL of 5% $NaNO_2$ was added to each test tube. After 5 min, 150 µL of 10% $AlCl_3$ was added. After further 6 min, 1 mL of 1M NaOH was added to the mixture. The solutions were diluted to a final volume of 5 mL with water and mixed. After mixing with distilled water, the absorbances of these prepared sample solutions were measured by UV/Visible spectrophotometer at 510 nm with respect to the blank solution. The total flavonoid content of the extract of stem bark of *Dalbergia Lanceolaria* L. was expressed as mg quercetin equivalent (QE) / g dry weight [21-24].

3. RESULTS AND DISCUSSION

The total flavonoid content of the stem bark of *Dalbergia Lanceolaria* L. was qualitatively and quantitatively investigated. Firstly, the phytochemical screening of the stem bark of *Dalbergia Lanceolaria* L. was performed. The results were shown in Table 1.

Table 1 Phytochemical Tests for *Dalbergia Lanceolaria* L.

No	Constituents	Reagent used	Observation	Results
1	Glycoside	10% lead acetate	White ppt	+
2	Flavonoid	conc HCl, Mg tuning	Pink color solution	+
3	Polyphenol	1% $FeCl_3$ + 1% $K_3[Fe(CN)_6]$	Greenish blue colour solution	+
4	Phenolic	10% $FeCl_3$	Greenish blue colour solution	+
5	Sugar	Benedict's solution	Orange-red ppt	+
6	Saponin	$NaHCO_3$	Frothing	+
7	Tannin	10% $FeCl_3$ dil H_2SO_4	Yellowish brown ppt	+
8	Terpene	$CHCl_3$, H_2SO_4 (conc) $(CH_3CO)_2O$	Pink color solution	+
9	Alkaloid	Wagner's solution	Reddish brown ppt	+
		Dragendroff's solution	Orange ppt	+
10	Lipophenol	0.5 N KOH, NaOH	Deep colour solution	+

(+) = presence (–) = absence

According to the resulted data, the stem bark of *Dalbergia Lanceolaria* L. consists of glycoside, flavonoid, polyphenol phenolic, sugar, saponin, tannin, terpene, alkaloid and lipophenol respectively.

The mineral contents of selected sample were described in Table 2.

Table2. Mineral Content in the Stem Bark of *Dalbergia Lanceolaria* L.

No	Elements	Symbols	Result (%)
1.	Calcium	Ca	1.55800
2.	Potassium	K	1.19400
3.	Chlorine	Cl	0.89200
4.	Silicon	Si	0.50160
5.	Phosphorus	P	0.05201
6.	Aluminium	Al	0.04230
7.	Iron	Fe	0.03569
8.	Sulphur	S	0.01271
9.	Titanium	Ti	0.00819
10.	Zinc	Zn	0.00728
11.	Strontium	Sr	0.00543
12.	Manganese	Mn	0.00478
13.	Vanadium	V	0.00351
14.	Rubidium	Rb	0.00112
15.	Bromine	Br	0.00101

As described in Table 2, the mineral contents of the stem bark of *Dalbergia Lanceolaria* L. were determined by EDXRF method. The stem bark of *Dalbergia Lanceolaria* L. consists of Ca, K, Cl, Si, P, Al, Fe, S, Ti, Zn, Sr, Mn, V, Rb and Br, respectively. According to these results, this selected sample was found to have the relatively high amount of calcium (Ca), potassium (K), Chlorine (Cl) and silicon (Si) and a fair amount of phosphorus (P), aluminium (Al), iron (Fe), and sulphur (S), respectively.

Moreover, the extract obtained from the stem bark of *Dalbergia Lanceolaria* L. with 95% EtOH was examined by using the special qualitative tests of flavonoid. The resulted data are tabulated in table 3.

Table 3 The Results of Qualitative Test for Flavonoid

No	Experiment	Observation	Inference
1.	Ferric Chloride Test:	Blackish red colour was appeared.	Flavonoid may be present.
2.	Shinoda's Test:	Red colour turn to pink.	Flavonoid is present.
3.	Lead Acetate Test:	Reddish brown bulky ppt was produced.	Flavonoid is present.

From these results, it was observed that the extract of the selected sample consists of flavonoid compounds.

The calibration curve was plotted against by using the resulting data of standard quercetin solution as shown in Figure 2.

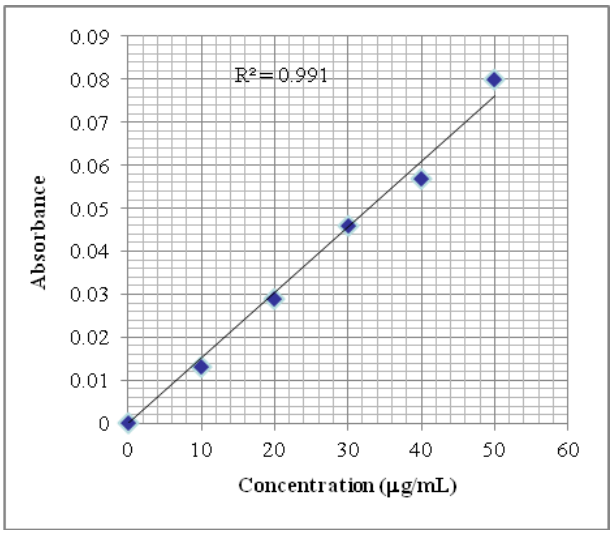

Figure2: Concentration absorbance calibration curve for standard quercetin

In addition, the total flavonoid content of the *Dalbergia Lanceolaria* L. was carried out by aluminium chloride spectrophotometric method using the quercetin as a standard. The absorbance values of prepared sample solutions were measured by UV/Visible spectrophotometer at 510 nm with respect to the blank solution. From these results, the amount of total flavonoid content of analyzed sample was obtained by using the standard graph. The results were described in Table 4.

Table 4 The Results of Total Flavonoid Content of Extract Solutions of *Dalbergia Lanceolaria* L.

Name of Sample	Flavonoid (mg/g)	Flavonoid (mg/g) Mean ± Standard Deviation
Dalbergia Lanceolaria L.	41.23	41.17 ± 0.11
	41.04	
	41.23	

The total flavonoid content present in the selected sample was found as 41.17 ±0.11 mg quercetin equivalents (QE) per gram dry weight.

4. CONCLUSIONS

In this research work, one of Myanmar well known plants, *Dalbergia Lanceolaria* L., Myanmar name Thit pagan which is flavonoid rich plant, was selected to qualify and quantify the flavonoid present in it. As mentioned in phytochemical test, the selected plant consists of glycoside, flavonoid, polyphenol phenolic, sugar, saponin, tannin, terpene, alkaloid and lipophenol respectively. The mineral contents of the stem bark of *Dalbergia Lanceolaria* L. were determined by EDXRF method. It was found that calcium (Ca), potassium (K), chlorine (Cl) and silicon (Si) contain the high amount. In accordance with the qualitative test of flavonoid, it was confirmed that this extract solution contains the flavonoid compounds. Moreover, the total flavonoid content of *Dalbergia Lanceolaria* L. was found to be 41.17 ± 0.11 mg quercetin equivalent (QE) per gram dry weight. The resulted data of the current study showed that the selected sample, the stem bark of *Dalbergia Lanceolaria* L., had the considerable amount of total flavonoid compounds. Flavonoid compounds that are secondary metabolites are antioxidants. Quercetin, the most abudant dietary flavano, is a potent antioxidant because it has all the right structural feactures for free radical scavening activity.

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