



Original Research Article

Screening and Quantification of Phytochemicals and Evaluation of Antioxidant Activity of *Albizia chinensis* (Vang): One of the Tree Foliages Commonly Utilized for Feeding to Cattle and Buffaloes in Mizoram

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ABSTRACT

The study was conducted for screening and quantification phytochemicals and to evaluate antioxidant activity of leaf extract of *Albizia chinensis* (Osb) Merr., known as 'Vang' in Mizoram. The plant is extensively utilised by dairy farmers for feeding to cattle and buffaloes in the state. The leaf samples were collected from three sub-divisions of Aizawl district of Mizoram, pooled together and three representative samples were drawn from each sub-division. Aqueous extracts were prepared using Soxhlet extraction system. On phytochemical screening, the leaf extract was found to be positive for polyphenols, flavonoids, terpenoids, saponins and quinone. The average polyphenols, condensed tannins, flavonoids, saponins and coumarin were estimated to be $7.87 \pm 0.81\%$ TAE, $0.18 \pm 0.05\%$ LE, 12.98 ± 0.47 mg RE/g, 5.82 ± 0.18 mg DE/g and 18.26 ± 0.38 mg CE/g respectively. The leaf extract of *Albizia chinensis* was also found to have Fe^{3+} to Fe^{2+} reducing activity (0.14 ± 0.00 mg Asc AE/g) and showed higher % inhibition ($83.69 \pm 0.52\%$) under ascorbate-iron (III) catalyzed phospholipid peroxidation. It was concluded from the study that *Albizia chinensis* i.e. Vang was rich in phytochemicals and possessed antioxidant properties which justified its usefulness for feeding to cattle and buffaloes in Mizoram.

Keywords

Albizia chinensis,
Phytochemical,
antioxidant,
Dairy animal,
Mizoram

Introduction

Dairy farming ensures livelihood to a major section of rural people in Mizoram. According to Directorate of Economics & Statistics, Govt. of Mizoram (2010), total cattle population was 34,988 comprising of 10,744 crossbred and 24,244 indigenous cattle, and total buffalo population was 5,832 in the state. Total milk production during 2011-12 was 13,950 tonnes and per capita availability of milk was 35 gm against

the ICMR recommendation of 240 gm of milk per individual per day indicating a huge gap between demand and availability of milk in the state (Economic Survey Mizoram, 2012-13).

Adequate supply of feed with appropriate quantities of nutrients as per requirement is must for realizing the true production potentiality of livestock. In Mizoram, the

nutritional needs of the dairy animals are met from crop residues and by-products, grasses, weeds and tree leaves gathered from uncultivated lands and nearby jungle (Kumaresan *et al.* 2010). The farmers utilize leaves of different tree species and these leaves become the only roughage source for animals during lean and rainy season of the year. The edible leaves of tree species are known to be rich in both nutrients and plant secondary metabolites known as phytochemicals. Animals get health benefits from the tree leaves both for nutritional and phytochemical constituents. Phytochemicals have protective and disease preventive properties and also act as anti-oxidants.

The *Albizia chinensis* (Osb) Merr., known as 'Vang' among Mizo people, is one of the commonly utilised tree species for feeding to dairy cattle and buffaloes in Mizoram. It is a large perennial tree found up to altitude of 1200 m above MSL in Mizoram. It belongs to the family *Mimosaceae* and commonly known as 'Siris' in other places (Swamlina, 2013). Although information are available about concentration of nutrients and mineral matters of *Vang* (Buragohain, 2014) and other tree species utilized for feeding dairy animals in Mizoram (Das *et al.*, 2006; Sarma *et al.*, 2007; Samanta *et al.*, 2009), information are scanty about the phytochemical composition and antioxidant properties. In the present study, therefore, an attempt was made for screening and quantification of phytochemicals and to evaluate the anti-oxidant properties of *Albizia chinensis* leaves known as 'Vang' in Mizoram.

Materials and Methods

Sample collection and preparation

The leaves samples of *Albizia chinensis* were collected from three sub-divisions of

Aizawl district of Mizoram namely, Tlangnuam, Thingsulthian and Aibawk. Healthy leaves representing different developmental stages were collected, air dried under shade and made in mesh with powdery consistency for preparation of plant extracts. Nine representative aqueous leaf extracts were prepared utilizing about 30 gm of dried leaves powder using Soxhlet extraction system.

Procedures for phytochemical screening

Alkaloids, flavonoids and polyphenols

Alkaloids, flavonoids and polyphenols were screened according to the procedure outlined by Akenga *et al.* (2005). For polyphenols, 1ml of extract was mixed with 2 ml of distilled water in a test tube followed by addition of few drops of 10% ferric chloride (FeCl_3). Appearance of blue or green colour was recorded as indicative of the presence of phenols. For flavonoids, 1 ml of plant extract was mixed with a few drops of dilute sodium hydroxide. An intense yellow colour produced in the plant extract, which became colourless on addition of few drops of dilute acid was assumed as the indicative of the presence of flavonoids. For alkaloid, about 0.2 g of plant material was heated with 2% H_2SO_4 solution for two minutes and then was filtered. To the filtrate a few drops of Dragendorff's reagent was added. An orange red precipitate was assumed as the indication of presence of alkaloids.

Glycosides and saponins

Glycosides and saponins were screened according to methods described by Sofowora (1982). For glycosides, in a test tube 5 ml of extract was treated with 2 ml of glacial acetic acid containing a drop of ferric chloride solution. Then it was underplayed with 1 ml concentrated sulphuric acid. A

brown ring at the interface indicated a deoxy sugar characteristic of cardenolites. For saponins, the extract was diluted with 20 ml distilled water and was agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam indicated the presence of saponin.

Terpenoids and phlobatannins

Terpenoids and phlobatannins were investigated according to methods of Edeoga *et al.* (2005). For terpenoids, in a test tube 5 ml of plant extract was mixed with 2 ml of Chloroform. 3ml of concentrated sulphuric acid (H₂SO₄) was then added to form a layer. A reddish brown precipitate at the interface indicated the presence of terpenoids. For phlobatannins, 1 ml of aqueous plant extract was boiled with 2% HCl solution which gives a red precipitate and was indicative of the presence of phlobatannins.

Quinone

To test quinone, 1ml of extract was added to 1ml of concentrated sulphuric acid in a test tube. Formation of red colour indicated the presence of quinones.

Reducing sugar

To test reducing sugar, One ml plant extract was boiled with a few drops of Fehling's solution A and B for a minute. An orange red precipitate indicated the presence of reducing sugars.

Procedures for quantitative estimation of phytochemicals

Total phenol

The total phenolic contents in the extracts were measured using Folin-Ciocalteu reagent method (Makkar *et al.*, 1993).

Aqueous extract of 0.02 ml, 0.05 ml and 0.1 ml were transferred into test tubes and the volume was made up to 0.5 ml with distilled water. To this solution, 0.25 ml of Folin-Ciocalteu reagent (1N) and then 1.25 ml of 20% sodium carbonate was added, and the tubes were vortexed thoroughly. Absorbance was recorded at wavelength of 725 nm using UV- visible spectrophotometer after 40 minutes. The concentration of total phenolic compounds in the extract was calculated as tannic acid equivalent (TAE) from standard curve. The total phenolic content was expressed as % on dry matter (DM) basis.

Flavonoids

Total flavonoid content of the extracts was determined according to method of Nabavi *et al.* (2008). The aqueous plant extract (0.5 ml) was mixed with distilled water (2 ml) and subsequently with 5% NaNO₂ solution (0.15 ml). After 6 mins of incubation, 10% AlCl₃ solution (0.15 ml) was added and then allowed to stand for 6 min, followed by addition of 4% NaOH solution (2 ml) to the mixture. Then, distilled water was added to the sample to make up the volume to 5 ml and the mixture was thoroughly mixed and allowed to stand for another 15 min. The mixture's absorbance was determined at 510 nm using UV-Visible spectrophotometer. The calibration curve was prepared using 100-1000 µl aliquots of rutin solution, 500 µl of acetic acid solution, 2 ml of pyridine solution and 1 ml of reagent aluminium chloride solution. The test was performed in triplicate and the flavonoid content was expressed as mg of rutin equivalent per g of extract (mg RE/g).

Saponin

Total saponin was determined according to method described by Makkar *et al.* (2007). A known quantity of freeze-dried extract

was dissolved in aqueous 50% methanol and a suitable aliquot (5 mg/ml) was taken. Vanillin reagent (0.25 ml; 8%) was added followed by sulphuric acid (2.5 ml; 72% v/v). The reaction mixtures was mixed well and incubated at 60°C in a water bath for 10 min. After incubation, the reaction mixtures were cooled on ice and absorbance at 544 nm (UV-visible spectrophotometer) were read against a blank that does not contain extract. The standard calibration curve was obtained from suitable aliquots of diosgenin (0.5 mg/ml in 50% aqueous methanol). The total saponin concentration was expressed as mg diosgenin equivalents (DE) per g dry weight (DW).

Proanthocyanidins (Condensed tannins)

Proanthocyanidin content (condensed tannins) was determined using the butanol-HCl assay as described by Porter *et al.* (1986). Butanol-HCl reagent (3 ml; 95:5 v/v) was added to the extract (0.5 ml) followed by ferric reagent (0.1 ml; 2% ferric ammonium sulphate in 2 N HCl), mixed thoroughly and incubated in a boiling water bath for 1 h. After the incubation period, absorbance at 550 nm was recorded using a UV-Visible spectrophotometer. A blank for each extract contained the sample, butanol-HCl reagent and ferric reagent, but without heating. The assay was done in triplicate. The concentration of proanthocyanidins (condensed tannins) (%) was expressed as leucocyanidin equivalents (LE) using the formula described by Porter *et al.* (1986) as follows:

Proanthocyanidins (%): $(A_{550} \times 78.26 \times \text{Dilution factor})$

Where A_{550} is the absorbance at 550 nm and the dilution factor was 1.0 for all the extracts.

Coumarins

Coumarin content was estimated following method outlined by Osorio and Martins (2004). To 500 µl of plant extract, 2 ml distilled water and 500 µl of lead acetate (5%, w/v) solution were added in a test tube. After shaking thoroughly, 7 ml of distilled water was added and mixing well, 2 ml of this solution was taken in another test tube and 8 ml of 0.1 M (v/v) hydrochloric acid solution was added. The solution was kept for 30 minutes at room temperature and absorbance was recorded at 320 nm using UV-Visible spectrophotometer. The total coumarin content was expressed as mg of coumarin equivalents per gm of sample extract (mg CE/g).

Procedures for measuring anti-oxidant activity

Iron (III) to iron (II) reducing activity

The ability of the tree foliage to reduce iron (III) was assessed by the method described by Oyaizu (1986). The aqueous extract of 160 µl was mixed with 0.84 ml of phosphate buffer (0.2 M, pH 6.6) to make the final volume 1 ml. Then, 0.1 ml of 1% aqueous potassium hexacyanoferrate solution was added. After 30 minutes of incubation at 50°C, the aliquot was added with 0.5 ml distilled water and 0.1 ml of aqueous FeCl₃ solution and the absorbance was recorded at 700 nm. The data were presented as ascorbic acid equivalents (Asc AE) in mg/g of sample.

Ascorbate -iron (III) - catalyzed phospholipid peroxidation

The ability of leaves to scavenge hydroxyl radicals was determined by the method as Aruoma *et al.* (1997). Mouse liver sample was mixed (1:10) with 10 mM phosphate

buffered saline (PBS, pH 7.4) and sonicated in an ice bath for preparation of homogenate. To 0.1 ml of plant extract 0.2 ml liposomes, 0.5 ml PBS and 0.1 ml of 1 mM FeCl₃ solution were added. Peroxidation was initiated by adding 0.1 ml of 1 mM ascorbic acid. The mixture was incubated at 37⁰ C for 60 minutes. The reaction was stopped by adding 1 ml of 10% trichloroacetic acid (TCA) and was centrifuged at 2000 rpm for 10 minutes. After centrifugation, 1 ml of 0.67% 2-thiobarbituric acid (TBA) in 0.05 M NaOH was added to the supernatant. The mixture then vortexed and heated in a water bath at 100⁰ C for 20 minutes. After cooling, 1 ml distilled water was added and absorbance was taken at 532 nm using UV-Visible spectrophotometer. The percentage inhibition was calculated where the control contained all the reaction reagents except sample.

Results and Discussion

The aqueous leaves extract of *Albizia chinensis* (Osb) Merr. showed the presence of polyphenols, flavonoids, terpenoids, saponins and quinone, but was negative for alkaloids, glycosides, reducing sugar and phlobatanins (Table 1). In the present study, total phenolics, condensed tannin, flavonoids, saponins and coumarin content of *Albizia chinensis* leaves were estimated (Table 2). The total phenolics content was found to be 7.87±0.81% TAE and CT as leucocyanidin equivalent was 0.18±0.05% on DM basis. The average flavonoids, saponins and coumarin content were estimated as 12.98±0.47 mg RE/g, 5.82±0.18 mg DE/g and 18.26±0.38 mg CE/g dry sample respectively.

The measurement of antioxidant activity of the leaf extract of *Albizia chinensis* are presented in table 3. The Iron (III) to iron

(II) reducing activity was found to be 0.14±0.00 mg Asc AE/g and % inhibition under the ascorbate-iron (III) catalyzed phospholipid peroxidation was 83.69±0.52%.

The genus *Albizia* comprises approximately 150 species mostly trees and shrubs native to tropical and subtropical regions of Asia and Africa (Kokila *et al.*, 2013). In India, *Albizia* species are valuable for producing high quality timber and gum and well known for medicinal properties of their bark and leaves (*A. Julibrissin*, *A. lebbeck*, *A. procera* and *A. amara*). *A. lebbeck* is known for its astringent property and also used to treat boils, cough, lung problem etc. Different species belonging to genus *Albizia* are known to contain different classes of secondary metabolites like saponins, terpenes, alkaloids and flavonoids. In the present study, *Albizia chinensis* was also found to contain polyphenols, flavonoids, terpenoids, saponins and quinone. Chulet Rahul *et al.* (2010) reported the presence of flavonoids, tannins and saponins in *Albizia lebbeck* leaves and absence of steroids. Mousallamy (1998) and Ueda *et al.* (2003) reported the presence of flavonoids and saponins respectively in *Albizia lebbeck* leaves. De Assis *et al.* (1999) isolated two macrocyclic spermine alkaloids from the leaves of *Albizia inopinata*.

The effects of tannins may be either beneficial or harmful depending on the type of tannin consumed, its chemical structure and molecular weight, the amount ingested, and the animal species involved. Low to moderate concentrations of tannins may improve the digestive utilisation of feed mainly due to a reduction in protein degradation in the rumen and a subsequent increase in amino acid flow to the small intestine (Robertson *et al.*, 1995; Butter *et al.*, 2000). Acceptability of *Albizia chinensis*

leaves readily by the dairy animals might be for its lower tannins content and low CT level (Table 2) both of which are often known for reducing the digestibility of nutrients of feeds including protein in animals.

Flavonoids has several biological functions including defense against pathogens and possess pharmacological properties such as antioxidant, anti-inflammatory, anti-thrombogenic, antimicrobial, anticancer,

antidiabetic and hypocholesterolemic activities. Saponins also have many health benefits viz. reducing blood cholesterol level, lowering cancer risk and bone loss and as immunity booster. Saponins can fight against parasites and help immune system to protect body against viruses and bacterial infections. Presence of flavonoids and saponins in *Albizia chinensis* justifies its importance as valuable feed for animals with medicinal values.

Table.1 Phytochemical screening in aqueous leave extract of *Albizia chinensis*

attribute	Polyphenols	Flavonoids	Terpenoids	Saponins	Alkaloids	Reducing sugar	Glycosides	Quinone	phlobatanins
<i>Albizia chinensis</i> (Os) Merr. (Vang)	+	+	+	+	ND	ND	ND	+	ND
The name within the parenthesis is the 'Mizo name' of the plant. + Presence of constituent, ND – not detected.									

Table.2 Quantification of phytochemicals in aqueous leave extract of *Albizia chinensis*

Attribute	Total Phenolics (% TAE on DM basis)	Condensed tannins (CT) as LE (% on DM basis)	Flavonoids (mg RE/g)	Saponins (mg DE/g)	Coumarin (mg CE/g)
<i>Albizia chinensis</i> (Os) Merr. (Vang)	7.87±0.81	0.18±0.05	12.98±0.47	5.82±0.18	18.26±0.38
The name within the parenthesis is the 'Mizo name' of the plant					

Table.3 Antioxidant properties of leave extract of *Albizia chinensis*

Anti-oxidant property	Iron (III) to iron (II) reducing activity [mg Asc AE/g]	Ascorbate –iron (III) catalyzed phospholipid peroxidation [% inhibition]
<i>Albizia chinensis</i> (Os) Merr. (Vang)	0.14±0.00	83.69±0.52

Fig.1 The Vang (*Albizia chinensis* (Osb) Merr.)



Coumarins are widely distributed in plants and are lactones of *O*-hydroxy-cinnamic acid derived from trans-cinnamic acid via oxidation-reduction and isomerisation to produce 1, 2-benzopyrone. The coumarin content was estimated to be 18.26 ± 0.38 mg CE/g of dry sample of *Albizia chinensis*.

Phenolic compounds are known to be responsible for antioxidant activity of plant (Rice-Evans *et al.*, 1996) by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals (Pokorny, 2001). Reducing power assay is often used to evaluate the ability of an antioxidant to donate an electron. The Fe^{3+} reduction is an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action (Yildirim *et al.*, 2001). Plant flavonoids are important natural phenolics (Agrawal, 1989) and act through scavenging or chelating process (Kessler *et al.*, 2003). In the present study, iron (III) to iron (II) reducing activity was found to be 0.1445 ± 0.00 mg Asc AE /g) and the presence of flavonoids in *Albizia chinensis* might also be the indication of its potentiality to act as antioxidant in animal system.

The non-sugar part of saponins has direct anti-oxidant activity which may result benefit like reduced risk of cancer and heart

disease. The % inhibition under the Ascorbate –iron (III) catalyzed phospholipid peroxidation was found to be $83.69 \pm 0.52\%$ which might be the indication that *Albizia chinensis* might contain some compounds which were having antioxidant activity responsible for higher % of inhibition.

It was concluded from the present study that leaves of *Albizia chinensis* are good sources of phytochemicals such as polyphenols, saponins, flavonoids and coumarin and possess antioxidant properties which justify its suitability as a valuable animal feed, particularly as feed for the dairy cattle and buffaloes in Mizoram.

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