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Pharmacognostic and Physicochemical evaluation of stem bark of *Acacia pennata (*L.) Willd., a folk plant of the Dimasa tribe of Assam.

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Bark of *Acacia pennata* is used in preparation of starter cakes among the Dimasa community of Assam state. The present study attempts to evaluate the pharmacognostical and physicochemical parameters of *Acacia pennata* (L.) Willd. The transverse section of the stem revealed an epidermis externally subtended by trichomes, a crashed cortical region due to massive secondary growth and large stellate pith. The physico-chemical parameters were evaluated- loss on drying (7%), total ash content (9.3%), Sulphated value of ash (2.5%), extractive value of bark powder with petroleum ether, ethanol, water and methanol (2.5%, 15%, 9% and 10% respectively). The Hausner ratio and Carr's indexes (1.43 and 30% respectively) indicate a poor flow. The bark powder is more soluble in alcohol (15%) and least soluble in petroleum ether (2.5%). Further studies on grain character of bark powder will reveal the feasibility for microbial substrate and evaluation of microflora in starter cakes is necessary to assess toxicity and quality of the finished products.

Keyword: Dimasa tribe, *Acacia pennata*, Pharmacognostic and Physico-Chemical Characters.

1. Introduction

Acacia pennata (L.) Willd**.,** belonging to the family Mimosaceae, is a large woody prickly climber with bipinnate leaves. Their flower is pale white and arranged in terminal leafy panicles. *Acacia pennata* is reported to have some medicinal value like the leaf juice when mixed with milk is given to the infant for indigestion. Leaf is sometime chewed with sugar and cumin in bleeding gum whereas the juice of bark is considered as an antidote for snake poison[4].

Acacia pennata is a medicinal and cultural important plant among the Dimasa community of Assam state. Stem bark of the plant is being used, since time immemorial, as a substrate for preparing rice starter cake or *Humao* by the Dimasas and claimed to be effective in producing alcoholic beverage of unique aroma and taste.

Fig1: Stem of Acacia pennata

Alcoholic beverage is produced by fermenting cooked rice, usually glutinous variety, with *Humao*. Traditionally, *Humao,* are prepared by pounding dried bark of *Themra* (*Acacia pennata*) and mixed with pre-soaked grounded rice. The mixture are moistened and made into round, flat cakes which are dried in the sun for 2-5 days (depending upon the prevailing temperature) and then used for fermenting cooked rice to produce rice-beer locally referred as *Judimah*. The latter is a popular drink among the Dimasas consumed in daily life as well as during socio-religious occasions. Sometimes spirit liquor called *Juharao* is produced by distillation of *Judimah* with crude still. It may be mentioned that both types of liquor, is a common drink of the Dimasas and have cultural significance as it is customarily used during rituals and festivals. There are a few reports on use of plants in preparation of starter cakes among different tribes [15], yet the pharmacognostic study of the plants has not been undertaken before. Considering the importance and cultural values of *Themra*, a need was felt for scientific investigations and study of various pharmacognostic and physico-chemical parameters of *A. pennata* which form the objectives of the present study.

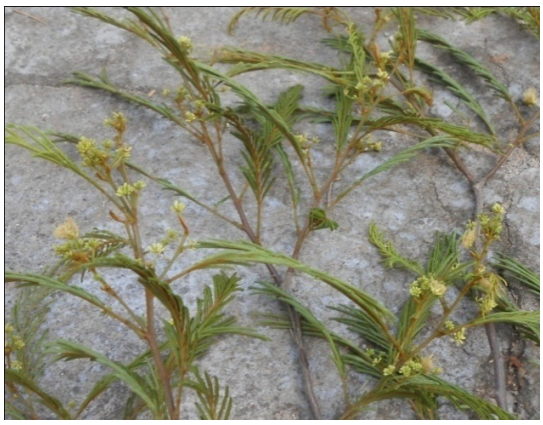

Fig 2: Flowering twig of *A. pennata*

The Dimasas are also referred as the hills Kachari, currently they are mainly distributed in Assam, Mizoram, Nagaland and Meghalaya states. Ethnically, the Dimasas are mongoloid and linguistically belong to the Bodo group in Assam-Burmese branch of Tibeto-Chinese family[14]. They were originally apart of the Bodo Kachari, who once ruled the entire Brahmaputra valley with its capital at Dimapur until the middle of the ten century. Following attack from the Ahoms, they shifted their capital to Maibong in present Dima Hasoa district of Assam.

2. Materials And Methods

2.1 Collection of Plant Material And Identification:

The stem bark of *Acacia pennata* (L.) Willd. (family Mimosaceae) was collected in the month of January, 2013 from local market. The plant along with flowers was collected from Dhansiri reserved forest, Assam and its botanical identity was confirmed by the first and fourth authors [2]. The specimen was compared with voucher specimen deposited in the Department of Life Science and Bioinformatics, Assam University, Diphu Campus.

2.2 Drying and Processing:

The stem bark was washed properly with deionised water to remove dust and dirt. The bark were dried by oven drying for about one week at 72^0C and then grounded into a coarse powder with the help of a blender. The grounded powder was sieved in a mesh of size 150 micron and the powder was used for determination of physico-chemical parameters and preparation of various solvent extracts. The fresh barks were used for macroscopic and microscopic observations.

2.3 Pharmacognostic Studies:

Morphological studies i.e. macroscopic and microscopic parameters were carried out by using simple determination technique. Organoleptic studies like shape, size, color, odor etc, were recorded (Table 1). The anatomical section of the stem bark was examined microscopically and photographed with the help of Trinocular Olympus Research microscope with photo micrographic attachment. Behaviour pattern of powdered stem bark on treatment with different chemical were also studied (Table 2).

2.4 Determination of Total Physico-Chemical Parameter:
The oven dried stem bark was subjected for determination of physico-chemical parameters to evaluate the percentage of total ash, water soluble acid insoluble ash [11, 16]. Hydration capacity, swelling index, flow properties, Loss on drying, pH, etc were also examined[1,3,5,6,7,9,10,13].

2.5 Hydration Capacity:
The method of Kornblum and Stoopak (1973) was used. A 1gm of the powder samples was placed in each of two 15ml plastic centrifuge tubes and 10ml distilled water was added from a 10ml-measuring cylinder and then stoppard. The contents were mixed on a vortex mixer for 2min. The mixture was allowed to stand for 10 min and immediately centrifuged at 3000 rpm for 10 min. The supernatant was carefully decanted and the sediment weighed. The hydration capacity was taken as the ratio of the weight of the sediment to the dry sample weight (g/g).

2.6 Swelling Index:
About 1 gm of stem bark powder was accurately weighed and transferred to a 100ml measuring cylinder and stoppard. The initial volume of the powder in the measuring cylinder was noted. The volume occupied by the sediment was noted after 24h. Swelling capacity was expressed and computed according to the following equation:

$$SI = (Xt - Xo) / Xt \times 100$$

Where SI is the % swelling capacity, Xt is the volume of the hydrated or swollen material and Xo is the volume of the material prior to hydration.

2.7 Moisture Sorption Capacity:
One gram of sample was accurately weighed and evenly distributed over the surface of a 70 mm tarred Petridish. The sample were then placed in a large desiccator containing distilled water in its reservoir (RH = 100%) at room temperature and the weight gained by the exposed samples at the end of a five day period was recorded and the amount of water sorbed was calculated from weight difference.

2.8 Flow Properties:
2.8.1 Bulk and Tapped Density: A 10g quantity each of the powder sample was placed in a 50 ml clean, dry measuring cylinder and the volume Vo, occupied by each of the samples without tapping was determined. After 500 manual taps, occupied volumes, V500 were determined. The bulk (pB) and tapped densities (pT) was calculated as the ratio of weight to volume (Vo and V500 respectively).

2.8.2 Hausner Ratio: The Hausner ratio is considered as indirect measurement of powder flowability and is indicative of interparticle friction. It was calculated by the formula given below, where ρB is the freely settled bulk density of the powder and ρT is the tapped density of the powder. Values less than 1.25 indicate good flow and a value greater than 1.25 indicates poor flow.

$$H = \frac{\rho T}{\rho B}$$

2.8.3 Carrs index: The Carr index is an indication of the compressibility of a powder. It was calculated by the following formula, where V_B is the freely settled volume of a given mass of powder, and V_T is the tapped volume of the same mass of powder. The value below 15% indicates good flow characteristics and a value above 25% indicates poor flow characteristics.

$$C = 100 \frac{V_B - V_T}{V_B}$$

2.8.4 Loss on Drying: A 2 g crude powder of bark was taken in previously dried and tarred flat weighing evaporating dish and then dried in an oven at 105ºC till constant weight was obtained (upto three consecutive reading). The weight after drying was noted and loss on drying was calculated. The percentage was calculated on the basis of sample taken initially.

2.8.4 pH of Aqueous Extract: The powdered materials (150 micron mesh size) were suspended in glass distilled water. After 2 hrs, filtered and the filtrate/clear solution was measured for pH.

2.8.5 Total Ash Value: Two grams powder of bark was taken in a silica crucible and ignited it by gradually increasing the heat to 500°C until it was white, indicating the absence of carbon. Ash was cooled in a desiccator and weighed without delay. Total ash value was expressed as percentage with reference to the sample taken initially.

2.8.6 Sulphated Value: The sulphated ash test is an analytical test for determining the inorganic content of a sample by weight. The ash powder was moistened with 1 ml of H_2So_4 and ignited to 800 $\pm$ 25ºC until it reaches a constant weight.

2.9 Determination of Alcohol Soluble Extractive:
Four grams of crude powder was macerated with 100ml of ethanol in a closed flask and kept on a rotary shaker for 24 h. Thereafter, it was filtered and 25 ml of the filtrate was evaporated to dryness at 105ºC for 2 h till constant weight was obtained. The percentage of extractable matter was calculated with reference to the sample taken initially. Further, the colour of the filtrate was also noted.

2.10 Determination of Water Soluble Extractive: Four grams of crude powder was macerated with 100ml of water in a closed flask and kept on a rotary shaker for 24h. It was then filtered and 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and dried at 105ºC for 2h and weighed. The percentage of alcohol soluble extractive was calculated with reference to the air-dried powered plant material taken initially. The colour of the filtrate was also noted.

2.11 Determination of Methanol Soluble Extractive: Four grams of crude powder was macerated with 100ml of methanol in a closed flask and kept on a rotary shaker for 24h. The mixture was filtered and 25ml of the filtrate was evaporated to dryness at 105ºC for 2h till constant weight was obtained. The percentage of extractable matter was calculated with reference to the sample taken initially. Additionally, the colour of the filtrate was also noted.

2.12 Determination of Petroleum Ether Soluble Extractive: Four grams of crude powder was macerated with 100ml of petroleum ether in a closed flask and was kept on a rotary shaker for 24 h. Then, it was filtered and the filtrate was evaporated to dryness at 105ºC for 2h till constant weight was obtained. The percentage of extractable matter was calculated with reference to the sample taken initially. Further, the colour of the filtrate was also noted.

3. Result and Discussion

3.1 Morphological evaluation of Stem Bark of *A. pennata*:

The organoleptic studies indicates the important of characteristic such as tongue sensitizing aromatic taste with pleasant mild sweetness, aromatic odour, stem bark powder when macerate with water have a rotten smell. The shape of stem bark is hard and woody and show sign of shrinkage on drying.

3.2 Microscopical Evaluation of Stem Bark of *Acacia pennata*:

The transverse section of the young stem revealed normal dicot structures. Epidermis is thick; the cells are small and compactly arranged and externally subtended by trichomes. Cortical region comprised of multilayered cells which has been crashed due to massive secondary growth. Secondary growth is extensive; secondary phloem is distributed in distinct patches while the wood is massive with large vessels. The pith is large and stellate.

3.3 Physico-Chemical Evaluation:

The physico-chemical parameters help in judging the purity and quality of the powder. The powder was evaluated for its physico-chemical parameters and found that loss on drying was 7% which is not too high, hence could discourage bacterial and fungal growth and favour long storage. Content of total ash was 9.3%; Sulphated value of ash was found to be 2.5%. The extractive value of the powder with petroleum ether,

ethanol, water and methanol were 2.5%, 15%, 9% and 10%. The flow properties of a powder are essential in determining its suitability as a direct compression excipient. The flow properties are also confirmed by Hausner ratio and Carr's index. Value less than 1.25 indicate good flow (20% Carr's index) and value greater than 1.25 indicate poor flow (33% Carr's index). In the present investigation both parameters were found to be 1.43 and 23% respectively, which indicate a poor flow. The bark powder is more soluble in alcohol (15%) and least soluble in petroleum ether (2.5%). Behaviour pattern of the stem bark on treatment with different chemical are shown in Table 2.

Table 1: Macroscopic characteristic of stem bark of *Acacia pennata*.

Constants	Features
Shape	Hard, woody
Size	3.87 cm thickness
Colour	Fresh stem bark deep grey, dried stem bark is greyish white
Surface	Presence of scars on nodal region, rough, sign of shrinkage on drying
Texture	Long and tough/woody
Odour	Characteristic, pleasant mild sweetness when fresh but odourless when dried.
Taste	Sweet juicy and tongue sensitizing

Table 2: Behaviour pattern of powdered stem on treatment with different chemicals reagent

Reagent	Observation
Powder as such	Greyish white
With acetic acid	Yellowish green
With conc. H_2so_4	Purplish brown
With conc.HNo_3	Brick red
With conc. HCl	Brownish black
Aq. NaOH (1N) solution	No change of colour

Table 3: Physicochemical parameter of stem bark of *Acacia pennata*.

Constants	Yield	Colour of filtrate
Hydration capacity g/g	4.125	-
Swelling index %	45	-
Moisture sorption capacity/g	1.20	-
Bulk Density g/ml	0.30	-
Tapped density g/ml	0.43	-
Hausner Ratio	1.43	-
Carr index %	30	-
Loss on Drying %	7	-
pH	7.33	-
Total ash %	9.3	-
Sulphated ash %	2.5	-
Alcohol soluble extractive %	15	Light yellow
Water soluble extractive %	9	Yellowish Brown
Methanol soluble extractive %	10	Grass green
Petroleum soluble extractive %	2.5	Light lemon green

4. Conclusion

The present investigation reported the physicochemical parameter of *Acacia pennata* and macroscopic and microscopic characters of the stem bark. Such study on the physicochemical parameters, organoleptic studies and behavioural pattern of powder provides important information which may be helpful in authentication and

adulteration for quality control of raw material. The use of bark for preparation of fermentation starter cakes has added to the existing knowledge of *Acacia pennata*. Further studies on grain character of bark powder are necessary to assess feasibility for microbial substrate. Investigation of microbial dynamics and their metabolites during different stages of fermentation is necessary to assess toxicity and quality of the finished products. Phytochemical screening of stem bark of *A. pennata* and nutritional assessment of alcoholic beer of the Dimasa community of Assam is in progress.

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Fig 3: TS of stem of *A. pennata*

Fig 4: Dried stem bark

Fig 5: *Humao* made of stem bark of A. *pennata*

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