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Phytochemical and biochemical study of four legume plants with detergent and anti-lice properties from the Eastern Himalayan region of India

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This study is aimed at the qualitative and quantitative investigation of the phytochemical content, macro- and micronutrients, proximate analysis and determination of antioxidant activities of four plants belonging to the family Leguminosae namely *Acacia pennata*, *Albizia lucidior*, *Albizia chinensis* and *Gymnocladus assamicus* widely used by the Adi tribe of Arunachal Pradesh for their surfactant and insect-repellent properties. The methanol extract of the seed pod of *G. assamicus*, the most popular soap-plant among the Adi people, showed maximum 1,1-diphenyl-2-picrylhydrazyl scavenging activity with EC_{50} of 13.50 µg/ml and presence of hitherto reported insecticidal metabolites like 2-hydroxy-gamma-butyrolactone and heptadecene-(8)-carbonic acid.

Keywords: Anti-lice, *Gymnocladus assamicus*, legumes, natural soap.

PLANT-based healthcare and cosmetic products are increasingly becoming popular because of the growing concern about the probable hidden adverse effects of synthetic products. For several thousand years plants have been used to treat human disorders, for relieving pain, as beautification products, as detergents as well as bathing soap and shampoo[1]. In Southeast Asia, herbal healthcare products are highly popular and the plant-based Indian *Ayurvedic* products have a considerable and continuously increasing market share around the world[2].

Arunachal Pradesh is the easternmost part of the Eastern Himalaya inhabited by 26 major and 110 sub-tribes. Due to geographical constraints and lack of modern amenities, majority of these ethnic population still largely depend on plant-based products in their daily lives. These people are a storehouse of oral traditional knowledge inherited from their forefathers about the medicinal and other useful plant resources native to this region. However, perusal of the literature has revealed that these endemic plant resources with traditional knowledge base

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have not yet been fully explored, which needs further validation at the next level for economic and livelihood security of mankind.

Belonging to the Leguminosae family, *Albizia lucidior* (Steud.) I.C. Nielsen, *Acacia pennata* (L.) Willd., *Albizia chinensis* (Osbeck) Merr. and *Gymnocladus assamicus* P.C. Kanjilal are the most commonly used as detergents and anti-lice agents in this hilly state of India. Among these four species, *G. assamicus* is prominently used by the tribes residing in East Siang, Lower Dibang Valley and Lower Subansiri districts of Arunachal Pradesh[3,4].

Locally known as *Dumkol* or *Tari*, *A. lucidior* is a large shrub or tree. The bark of this plant is crushed using a stone or any other hard object with the addition of small amount of water. The crushed bark is rubbed over the body which acts both as a soap and a body brush[3]. In addition, crushed bark, fruit and stem of this plant have been reported to be used as a fish poison in Nepal[5].

The shrubby species *A. pennata* (climbing wattle), locally known as *Ramgir* is a versatile source of components with bioactive properties[6]. Bark and the leaf of this plant are used as bathing soap by humans and for domestic animals[3,4].

Widely distributed in India, *A. chinensis*, locally known as *Tattong* or *Tatkung* is a large deciduous tree. It has been reported as a good source of timber, fuel, fodder, soil nitrogen fixer[7] and to possess cytotoxic[8] and antioxidant[9] activities. Bark of this plant has been used as soap/shampoo having anti-lice and anti-dandruff properties[3].

G. assamicus, locally known as *Minagmose* or *Dikang* among the *Adi* tribe, belongs to the subfamily Caesalpiniaceae. A critically endangered species endemic to Arunachal Pradesh, it is a medium sized deciduous tree (ca. 15 m) presently confined to the West Kameng, Tawang and Siang districts of the state. Different parts of this plant are used to treat stomach and skin disorders in humans as well as in domestic animals – pods as shampoo and soap, dry leaves in controlling soil-borne insects and roasted seeds are used as coffee substitute[10]. The fleshy ripe pods are used for washing clothes and cleaning traditional ornaments made of gold and silver. The pods are also used as shampoo to make the hair shiny[3,11]. In a recent study, Gupta *et al.*[12] evaluated the antioxidant properties of different parts of *G. assamicus*[12].

Continuing our studies of the medicinal plants from North East India[13–16], here we report the results of our investigation on the phytochemical, antioxidant, proximate and gas chromatography mass spectroscopic analyses carried out on the above-mentioned four traditionally useful plants.

The field and herbarium methods of Jain and Rao[17] were followed during field survey. *A. lucidior*, *A. pennata*, *A. chinensis* and *G. assamicus* were collected from East and West Siang districts of Arunachal Pradesh in their natural habitats. They were identified and authenticated at the Botanical Survey of India, Arunachal Regional Centre, Itanagar and voucher specimens for all the four plants were deposited.

The leaves, barks and pods of *G. assamicus* were collected from mature plants and washed thoroughly in running water to remove soil and other dust particles. Then the barks were cut into small pieces and shade-dried at room temperature. The dried samples were powdered in a Wiley mill and kept in a moisture-free container until further chemical analysis. For each plant, the powdered biomass (500 g) was soaked in ethanol (2500 ml) with occasional swirling at room temperature for 48 h. Then the extract was filtered through Whatman No. 1 filter paper and the solvent was evaporated under reduced pressure in a rotary evaporator to get 19.0 g (3.8%) of bark and 24.7 g (4.94%) of leaf of *A. pennata*, 42.0 g (8.40%) of bark and 45.1 g (9.02%) leaf of *A. lucidior*, 30.5 g (6.10%) of bark and 18.3 g (3.66%) of leaf of *A. chinensis* and 230.0 g (46%) of seed pod and 19.8 g (3.96%) of leaf of *G. assamicus.*

The presence of saponins, tannins, alkaloids, triterpenes, phenolics, flavonoids, steroids, glycosides, reducing sugars, gums and mucilages was detected using simple qualitative and quantitative methods of Trease and Evans[18] and Sofowora[19] respectively. Proximate analysis of the samples, i.e. moisture, crude fat, fibre, protein and ash was done following the respective protocols by Schanderl[20]; the values are presented as average of triplicate analysis. Proximal composition was evaluated following the procedure of Tadhani and Subhash[21]. Mineral content was determined by flame photometry (Model 405, Corning, Halstead Essex, UK) using NaCl and KCl to prepare standards. Minerals were analysed using the solutions obtained by dry ashing the samples at 55°C and dissolving them in 10% HCl (25 ml) and 5% lanthanum chloride (2 ml), boiling, filtering and making up to the standard volume with deionized water. Phosphorus was determined colorimetrically (Spectronic 20; Gallenkamp, London, UK) with KH_2PO_4 as standard. All other elements (Ca, Mg, Zn, Fe, Mn, Cu and Cr) were determined by atomic absorption spectrophotometry (Model 403; Perkin-Elmer, Norwalk, Connecticut, USA).

GC–MS analysis was carried out on a Perkin Elmer GC Clarus 500 system comprising a AOC-20i auto sampler and a gas chromatograph interfaced to a mass spectrometer (GC–MS). Sample volumes of 1 µl were injected in split-less mode. The GC column used for analysis was TG-5MS with an inner diameter of 0.25 mm, 30 m length and 0.25 µm film thickness. Helium was used as carrier gas at a flow rate of 1 ml/min. The extracted sample was analysed under the following oven temperature programme: injection at 250°C followed by 2°C/min oven temperature ramp to 70°C and then by 5°C/min to 250°C and finally with 24 min isothermal at 280°C. For all samples, the temperature programme was set according to Jiang *et al.*[22]. Mass spectra were acquired using full-scan

Table 1. DPPH radical scavenging activity and EC_{50} of the plants

Species	Extract	Concentration (μg/ml) 5.0	10.0	20.0	40.0	60.0	80.0	100.0	EC_{50} (μg/ml)
Acacia pennata	Acetone	17.17 ± 0.09	27.37 ± 0.01	44.67 ± 0.01	58.96 ± 0.02	63.53 ± 0.01	65.47 ± 0.01	66.95 ± 0.01	31.17
	Methanol	23.58 ± 0.01	37.46 ± 0.01	54.33 ± 0.02	63.86 ± 0.01	67.54 ± 0.01	69.33 ± 0.02	70.4 ± 0.12	20.82
	Water	15.03 ± 0.02	19.44 ± 0.01	28.44 ± 0.01	40.05 ± 0.02	49.63 ± 0.01	56.63 ± 0.09	60.43 ± 0.02	60.64
Albizia lucidior	Acetone	20.13 ± 0.01	25.36 ± 0.01	32.07 ± 0.01	36.04 ± 0.01	41.65 ± 0.01	47.43 ± 0.01	51.06 ± 0.01	107.66
	Methanol	24.29 ± 0.01	37.61 ± 0.01	45.96 ± 0.01	49.61 ± 0.01	53.78 ± 0.01	55.21 ± 0.01	56.27 ± 0.02	43.65
	Water	14.35 ± 0.01	22.51 ± 0.01	29.51 ± 0.01	33.17 ± 0.01	39.29 ± 0.01	44.26 ± 0.01	47.12 ± 0.01	131.88
Albizia chinensis	Acetone	20.41 ± 0.01	25.33 ± 0.01	34.8 ± 0.06	46.43 ± 0.01	54.79 ± 0.01	60.68 ± 0.01	62.8 ± 0.06	46.10
	Methanol	24.08 ± 0.01	29.47 ± 0.01	38.67 ± 0.01	49.37 ± 0.01	57.39 ± 0.01	63.85 ± 0.01	65.33 ± 0.01	37.49
	Water	15.33 ± 0.15	20.58 ± 0.01	31.93 ± 0.02	40.29 ± 0.02	47.52 ± 0.09	54.3 ± 0.15	58.43 ± 0.01	64.94
Gymnocladus assamicus	Acetone	25.48 ± 0.01	43.44 ± 0.02	53.58 ± 0.02	60.24 ± 0.01	67.27 ± 0.01	71.98 ± 0.01	73.33 ± 0.01	18.95
	Methanol	28.07 ± 0.01	51.97 ± 0.01	58.03 ± 0.02	64.23 ± 0.01	73.21 ± 0.01	75.25 ± 0.01	77.23 ± 0.01	13.60
	Water	20.31 ± 0.01	30.93 ± 0.01	48.74 ± 0.03	55.91 ± 0.03	63.89 ± 0.03	66.65 ± 0.01	69.34 ± 0.01	28.20
Ascorbic acid		30.43	30.46 ± 0.03	54.27 ± 0.04	64.67 ± 0.03	69.71 ± 0.03	76.46 ± 0.03	78.05 ± 0.02	10.83

monitoring mode with a mass scan range of 40–650 m/z. The chromatogram and mass spectra were evaluated using the Xcalibur™ software embedded in the GC–MS/MS system.

The antioxidant activity of both the plant extracts and the standard was assessed on the basis of the radical scavenging effect of the stable 1,1-diphenyl-2-picryl-hydrazyl (DPPH)-free radical activity using a modified method[23]. The stock solution of the crude extract was prepared by dissolving a known amount of sample in methanol, acetone and water separately. From the stock, working solutions of different concentrations (5, 10, 20, 40, 60, 80 and 100 μg) were prepared. Ascorbic acid solution of identical strength was prepared and used as positive control. A solution of DPPH in methanol (0.004%) was prepared and used as the negative control. These were kept for incubation in the dark for 30 min following which the optical density of the mixture was measured at 517 nm using a spectrophotometer (Cecil-Elect, UK). From the calculated values, a graph was plotted for the determination of EC_{50} of the extracts.

The free-radical scavenging activity (RSA) of the extracts against DPPH was calculated according to the formula

$$\% \text{ RSA} = 100 \times \left(1 - \frac{A_E}{A_D}\right),$$

where A_E is the absorbance in the presence of the extract and A_D is the absorbance of the control in the absence of the extract.

All the chemicals, including the solvents used were of analytical grade and purchased from Merck, unless stated otherwise.

The data were expressed as mean ± SEM. Results obtained from these tests were analysed using the Origin 8.0 software. One-way analysis of variance (ANOVA) and the Tukey post hoc test were used as statistical tools to identify significant differences ($P < 0.05$).

The antioxidant activity is related to the free radical scavenging activities. Free radicals are responsible for the inflammation in case of injuries and infections, and are related to ageing in humans[24]. Table 1 presents the results of antioxidant activity studies of the four legume plants. All the extracts show significant radical scavenging activity increasing with concentration. For all the four plants, the methanol extract was found to possess maximum activity followed by the aqueous extract. The methanol extract of *G. assamicus* exhibited significant radical scavenging activity with close EC_{50} value close to that of standard ascorbic acid, validating its traditional use as a soft bathing soap for humans, including new-borns.

Different indicative tests for the respective phytochemicals were performed to confirm their presence in the studied plants. All the four plants were found to possess alkaloids, glycosides, saponins, phenols and tannins. Hager, Myer and Wagners' tests were performed for the detection of alkaloids. Presence of alkaloids in all the plant parts was indicated by at least two of these three tests in each case. Presence of glycosides was determined by Fehling, Benedict, Molisch and Barfoeds' tests, and all plant parts tested positive in at least two of these tests. Salkowski's test was carried out to detect the presence of steroids. These were not detected in the leaves of *A. pennata* and *G. assamicus*. Foam test showed the presence of saponins in each part of all the four plants. Ferric chloride and lead acetate tests indicated the presence of phenols in the plants. No flavonoid was detected in the leaves of *A. pennata*, *A. chinensis*, *G. assamicus* and barks of *A. lucidior* in lead acetate test. No free protein was detected in the bark of *A. lucidior* in the Biuret test; gums and mucilage were detected only in the leaf of *A. lucidior* and seed pod of *G. assamicus* in the alcohol

test. Ferric chloride test indicated the presence of tannins in all the plants.

Figure 1 shows a plot of proximate composition of the four plants. Compared to the bark, leaf shows higher proximate composition like free amino acid, crude protein, crude fibre, crude fat and ash content in all four species. *G. assamicus* seed pod was found to contain a notable amount of crude fibre (60%), followed by the leaf extract of *A. lucidior* (30%).

Figure 2 shows the carbohydrate content like starch, amylose, total sugar and reducing and non-reducing sugars of the plants. Among the four species, seed pod of *G. assamicus* contains the highest amount of total carbohydrate.

When applied, nutrients and antioxidants can be absorbed transdermally by the body[25]. As evident from Figure 3, the seed pods of *G. assamicus* contain the highest amount of P, Na and K minerals. *A. lucidior* leaves contain maximum Ca and Mg while *A. pennata* barks and leaves contain the highest amount of N. Figure 4 shows the micronutrient content of the plants. It can be seen that the *G. assamicus* seed pods possess a considerable amount of Fe, Mn and Cu. However, Zn-content of *G. assamicus* is very low; it is highest in the leaves of *A. lucidior*.

Figure 5 records the secondary metabolite content of the plants. It can be seen that the total saponin content of the barks of the first three plants and seed pods of *G. assamicus* is high. The bark of *A. pennata* and the seed pod of *G. assamicus* contain a considerable amount of alkaloids, total phenol, flavonoids and tannins. Except for tannin, *G. assamicus* seed pods contain the highest amount of all the secondary metabolites.

Table 2 shows the results of GC–MS studies carried out on all the four plants. It can be observed that they contain different fatty acids like dodecanoic acid, tetradecanoic acid, pentadecanoic acid, heptadecanoic acid and octadecanoic acid which are potential precursors of

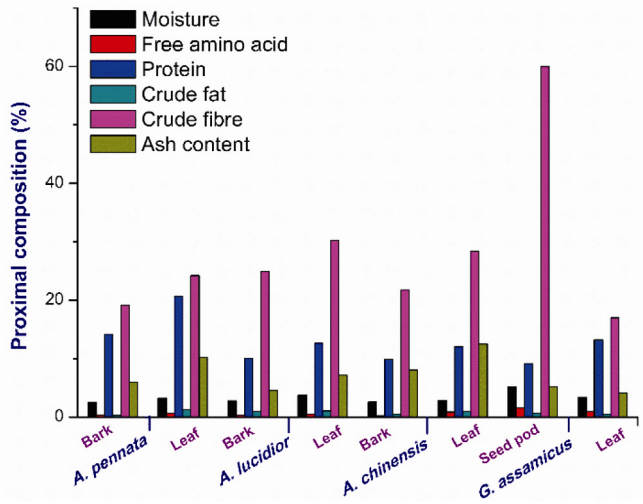

Figure 1. Proximate composition of the plants.

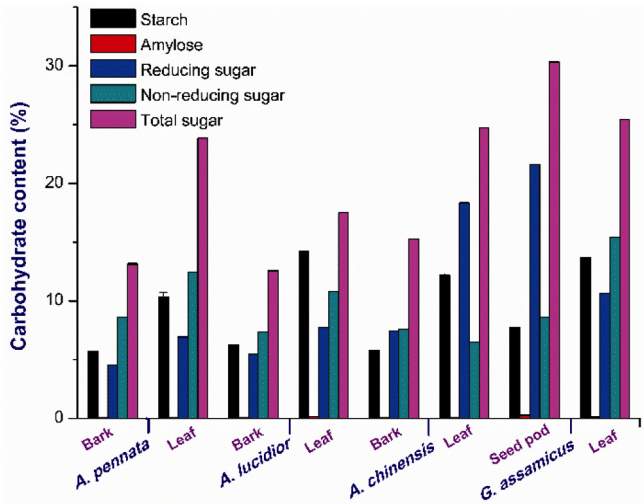

Figure 2. Carbohydrate content of the plants.

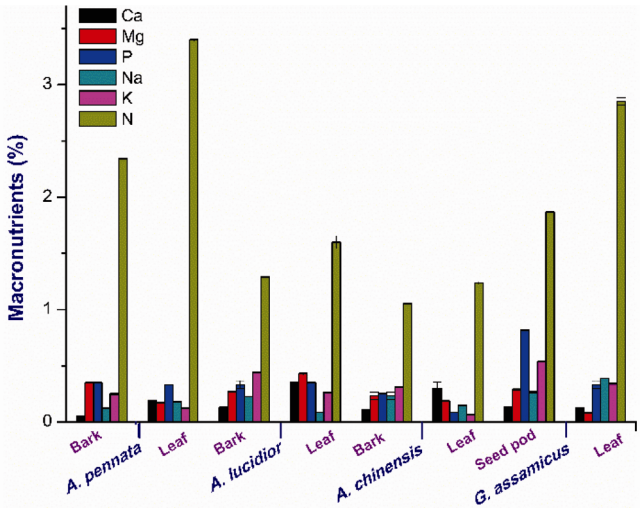

Figure 3. Macronutrient content of the plants.

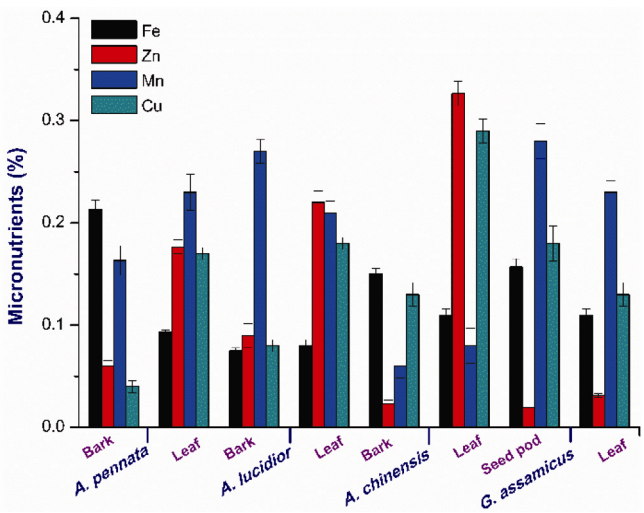

Figure 4. Micronutrient content of the plants.

Table 2. Compounds identified by GC–MS studies from the plants

Compound identified	Peak area (%)			
	A	*B*	*C*	*D*
1,2,3-Propanetriol	–	3.1	–	–
2-Hydroxy-gamma-butyrolactone	–	–	–	2.99
2,3-Dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one	–	1.87	1.93	1.02
5-Hydroxymethyl furfural	–	5.26	6.26	2.51
1,2,3-Propanetriol,1-acetate	–	0.46	0.49	–
Neric acid	–	1.83	1.74	1.54
1,7-Octadien-3-ol,2,6-dimethyl	2.9	2.39	2.39	0.29
Guanosine	–	6	–	–
1,6-Octadiene,3-ethoxy-3,7-dimethyl	–	–	–	17.75
Dodecanoic acid	2.78	0.7	0.54	–
4-((1e)-3-Hydroxy-1-propenyl)-2-methoxyphenol	0.9	0.69	0.75	–
Tetradecanoic acid	1.85	0.54	0.45	0.49
Mome inositol	–	1.42	2.1	27.89
2-Methyl-2-(4h-1,2,4-triazol-4-yl amino)propanenitrile	–	2.04	–	–
Pentadecanoic acid	8.26	0.32	0.31	9.25
Hexadecanoic acid, methyl ester	0.5	0.3	0.31	0.12
9-Octadecenamide	0.44	0.22	0.24	–
Heptadecanoic acid	–	0.37	0.38	0.22
Octadec-9-enoic acid	8.15	–	–	–
Cis-vaccenic acid	3.33	8.56	–	–
9,12-Octadecadienoic acid (*z*, *z*)	0.69	13.47	13.58	–
Cis-9-hexadecenal	–	–	9.12	–
Octadecanoic acid	2.7	5.54	5.64	2.37
Methyl octadeca-9,12-dienoate	2.08	–	–	2.11
Heptadecene-(8)-carbonic acid-(1)	–	–	–	13.49
10,12-Hexadecadien-1-ol	2.05	0.4	0.34	1.83
9-Octadecenamide	0.41	0.22	1.92	–
12-Hydroxy-9-octadecenoic acid	3.8	0.81	0.74	–
2,6-Dimethyltride canenitrile	–	0.49	0.53	–
9-Octadecenoic acid			1.02	
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	0.41	0.63	0.66	0.31
17-Octadecen-1-ol acetate	–	0.53	0.57	–
2-Hexadecyl-5-methylpyrrolidine	–	0.82	0.8	–
Chondrillasterol	3.29	2.71	2.75	–

A, *A. pennata*; *B*, *A. lucidior*; *C*, *A. chinensis*; *D*, *G. assamicus*.

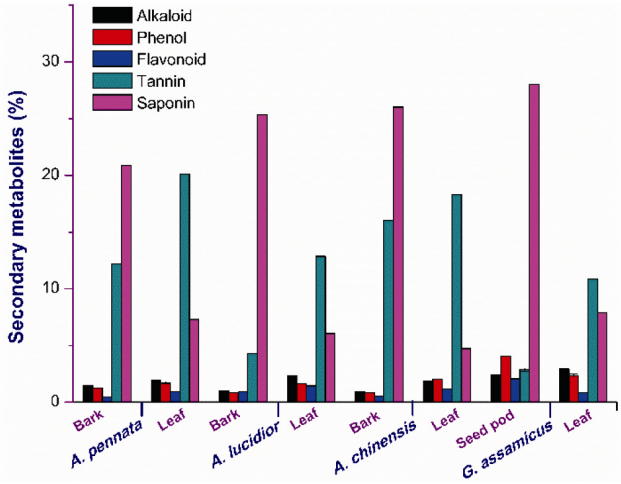

Figure 5. Secondary metabolite content of the plants.

soap-esters along with saponins like hexadecanoic acid methyl ester and hexadecanoic acid-2-hydroxy-1-(hydroxymethyl)ethyl ester. Compounds with insecticidal activities like 2-hydroxy-γ-butyrolactone and heptadecene-(8)-carbonic acid were also found to be present.

The seed pod of *G. assamicus*, the most popular soap material, contains the highest amount of saponin, total phenols and flavonoids (Figure 5). Presence of 2-hydroxy-γ-butyrolactone can be attributed to the flavour and better cleansing properties of *G. assamicus* seed pods. Tyrosinase activity and oxidative stress caused by reactive species like reactive oxygen and nitrogen species are the main contributors of melanogenesis resulting in skin pigmentation and ageing[26]. Controlling this oxidative stress may be an effective way of regulating melanogenesis. The presence of considerable amounts of flavonoids and phenolics in the plant extracts (Figure 5) may be related to their antioxidant potential (Table 1), and hence their popularity as beauty soaps for bright skin and shampoos for shiny hair. Glycerol (1,2,3-propanetriol), a common ingredient of commercially available beauty soaps was found to be present in the bark extract of *A. lucidior*.

In a recent study, 2,3-dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one (DDMP) has been found to exhibit strong antioxidant properties[27]. Except for *A. pennata*, DDMP is present in the other three plants. Compounds having γ-butyrolactone moiety in their structure have been reported to possess different bioactivities, including anti-fungal and antibacterial activities[28,29]. GC–MS studies indicated the presence of 2-hydroxy-gamma-butyrolactone in *G. assamicus*. Heptadecene-(8)-carbonic acid is a known natural insecticide[30] and *G. assamicus* contains around 13.49% of it substantiating its use as a lice-repellent and natural insecticide.

In conclusion, the preliminary phytochemical investigation along with the evaluation of antioxidant potential of four legume plants of Arunachal Pradesh was carried out. The four plants studied are considered as effective natural soaps for cleansing and beautification. The presence of considerable saponin content, different long-chain fatty acids, their esters and previously known insecticidal metabolites in the plants substantiates their traditional use as natural soaps.

Further studies on bioassay-guided isolation of active compounds and application of these plant extracts for the development of natural healthcare products are under way.

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