



Corresponding author
Ajuru, Mercy Gospel
Tel.: +2347036834588
ajurumercygospel@yahoo.com

DARSGAH-E-AHLEBAIT

Journal of biotechnological sciences (ISSN: 2308-0043)

Phytochemical and Proximate Compositions of the Leaf and Root of *Asystasia gangetica* (L.) T. Anderson in Rivers State

¹Ajuru M. G., ¹Nmom F. W., ¹Kpekot A. K., ¹Morris E. I., ¹Macfarlane O. M.

¹Department of Plant Science and Biotechnology, Rivers State University, Nkpulu-Oroworuokwo, P.M.B. 5080, Port Harcourt, Rivers state, Nigeria.

Abstract

The plant, *A. gangetica* is a rapidly growing perennial, shrubby herb of about 1m in height and can be found along roadsides, cultivated areas and river banks. It belongs to the family Acanthaceae and is a dicotyledonous flowering plant. Comparative study on the Proximate and phytochemical compositions of the root and leaf of *A. gangetica* (Chinese Violet) was carried out in the Department of plant Science and Biotechnology, Rivers State University, using standard methods. Data obtained in this study were subjected to one way analysis of variance (ANOVA). The results of the proximate composition showed highest quantity of carbohydrate, protein, lipid, and ash in the leaves of *A. gangetica* while the root was observed to have the highest amount of moisture. Results of phytochemical screening revealed the occurrence of tannin, saponin, phytate, flavonoid, alkaloid and cyanogenic glycoside in both the root and leaf of the plants. However, highest values of tannin (148.75 ± 0.00), saponin (5.61 ± 0.01), flavonoid (13.18 ± 0.00), alkaloid (15.93 ± 0.01) and cyanogenic glycoside (120 ± 0.00) were recorded for the leaf samples whereas the root sample was only highest in phytate content (19.43 ± 0.01). Generally, there is a significant difference between the phytochemical compositions of both parts of *A. gangetica* as the leaf recorded more phytochemical concentration than the root at $P \leq 0.05$. The results of this study indicated that *A. gangetica* is of nutritional, medicinal and veterinary importance considering its diverse uses for ethnopharmacological purposes in different parts of the world. Pharmaceutical industries could exploit these phytochemicals and use them for the production of drugs.

Keywords: Acanthaceae, *Asystasia gangetica*, Leaves, Phytochemicals, Proximate Composition, Roots

Introduction

Medicinal plants are a gift to us from nature as they provide a number of health benefits to us. In India, these medicinal plants are used for about centuries for their properties and are still used to this date. India has a variety of traditional medical systems like Ayurveda, siddha, unani and a huge class of ethnomedicine.

According to [1], 2003, over 80% of the people of developing countries are relying on the traditional medicines that are extracted from the plants for their primary health needs. Use of these traditional medicines for the preparation of modern medical preparations is indispensable and thus 'Phytomedicines' are a link between the traditional and modern medicine.

Proximate analysis is the partitioning of compounds in a feed into six categories based on the chemical properties of the compounds. It comprises of the mass percentages of moisture, ash, volatile matter, and fixed carbon which are obtained from a series of three standardized tests [2].

Vegetables are the cheapest and most available sources of important nutrients supplying the body with mineral salts, vitamins, and certain hormone precursors, protein, energy and essential amino acids therefore their proximate composition analysis is paramount [3, 4]. Proximate analysis of edible plant and vegetables plays an important role in assessing their nutritional significance.

The chemicals that are produced by plants are called phytochemicals. These are produced by the plant's primary and secondary metabolism. These phytochemicals are important for the plants to thrive or thwart other plants, animals, insects and microbial pests and pathogens. They also help plants and protect them from disease and damage caused by environmental hazards like pollution, UV, stress and draught. They are used as traditional medicine and as poisons from ancient days.

Asystasia gangetica is a species of the plant family Acanthaceae. It is commonly known as the Chinese violet, Coromandel or creeping foxglove and it may simply be called *Asystasia*. *A. gangetica* is an annual weed that grows upright and creeps up to a very thick thicket [5]. It has bushy and gnarled stem, each easily forming roots on the ground. The leaves are oval and dark green. The flowers are white with a purple mosaic on the petals [6]. The fruit is capsule shaped with size 13mm X 2mm and contains 2 - 4 seeds [7]. The crop is flowering and yielding seeds throughout the year [8]. In a shaded place, *A. gangetica* will show more vegetative organs; while in the open space, it will produce more flowers and seeds [5]. It takes *A. gangetica* within six weeks to flower and produce seeds. It is a weed of roadside, waste places, fields, farms, gardens etc [9].

A. gangetica is an invasive weed due to its ability to produce large quantities of seeds, estimate shows that it produce around 27 million seeds per hectare [5], thrown as far as 6m [9] and easily germinates so quickly on dormant lands. New plants can also grow from the stem when touching the ground [5]. *A. gangetica* is also able to grow well in low light conditions and low soil fertility (Samedani et al., 2013), even grow at a 90% shade level [9].

A. gangetica can also be used as a green manure for peanut plant [10] and as a medicinal plant [11, 12]. Trailing to erect, glabrous to lightly hairy, perennial herb to 3.3ft tall with weak stems, rooting from the nodes [5]. Leaves are opposite, simple, ovate, to 3 inches long and 1.5 inches wide, petiolate, margins entire to slightly toothed. Flowers purplish-blue (sometimes yellow or white with dark purple streaks), to 2 inches long, arranged along one side of a long terminal spike; subtended by 5 linear-lanceolate green sepals, petals hairy, fused at base to form a funnel-shaped tube with 5 rounded, spreading lobes [6]; 4 fertile stamen fruit is a hairy, club shaped capsule to 1cm long, containing 2-4 angular, gray seeds [5].

The plant is locally used as a pot herb and leafy vegetable. It is also often prepared in a mix with other leafy vegetables [13]. In Africa, an infusion of the plant is used to ease pain during childbirth [14] and the sap is applied to sores, piles and wounds in embrocations to treat stiff neck and enlarged spleen in children. Powdered roots are considered analgesic and used in treating stomach ache and snake bites. A leaf decoction is used as an analgesic and to treat epilepsy and urethral discharge. In Nigeria, the leaves are used to treat asthma [15]. In India, the sap is applied to swellings; it is also used as a vermifuge and to treat rheumatism. The juice, together with lime and onion juice is recommended for dry coughs with an irritated throat and discomfort in the chest. The leaves and flowers are used as an intestinal astringent. Plants are pounded with water to make a wash against fleas for young animals. A number of medicinal properties are attributed to different parts of this plant and research has shown that this plant can be consumed by humans. This study is therefore conducted to analyze the proximate and phytochemical constituents of the leaves and roots of *A. gangetica* with the objective of comparing the composition of the leaves and roots for taxonomic purposes.

Materials and Methods

Collection, Identification and Preparation of *A. gangetica* Leaves

The vegetative parts of *A. gangetica* were collected in the month of November, 2020 from Abua/Odual Local Government Area in Rivers State, Nigeria and conveyed to the Department of Plant Science and Biotechnology Rivers State University where it was identified and authenticated by Dr Mercy Gospel Ajuru, a Plant Taxonomist in the Department. The leaves were then separated from the stalk, washed and air dried at room temperature (24°C) for a period of 21 days and then pulverized, crushed into fine powder and weighed. Aliquot portions of the powdered leaves were weighed and used for proximate analysis.

Extraction of the Plant Leaves

Ethanol extract of the plant leaves was prepared by soaking 50g of the dry powdered plant leaves in 500ml of Distilled water and ethanol at room temperature in a sterile conical flask separately and stand for 3 days with intermittent shaking. The extract was filtered first using a Whatmann filter paper No. 42 (125mm) and then through cotton wool. The extract was concentrated using a rotary evaporator with the water bath at 70 °C for 3 hours. The crude extract was then stored at 4°C in a well labeled sterile conical flask. Aliquot portions of the crude plant extract residue were weighed and used for phytochemical screening

Proximate Analysis

The moisture content was determined by drying at 105°C in an oven until a constant weight was reached. For total ash determination, the leaves samples were weighed and converted to dry ash in a muffle furnace at 450 and at 550°C for incineration. The crude lipid content was determined by extraction with hexane, using a Soxhlet apparatus. All these determinations were determined using the standard methods of the Association of Official Analytical Chemists [16]. Kjeldahl method was used for crude protein determination. Carbohydrate content was determined by calculating the difference between the sums of all the proximate compositions and the total percentage composition. Crude fibre values were obtained by multiplying the carbohydrate, protein and fat by the Atwater conversion factors of 17, 17 and 37, respectively [17].

Phytochemical analysis

The phytochemical screening of the plants for various phytochemical compositions was conducted using standard procedures as described by [18] and [19].

Test for Saponins

0.5g of extract was added to 5ml of distilled water in a test tube and the solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for Tannins

Two methods were used to test for tannins: (a) To 10ml of freshly prepared 10% KOH in a beaker, 0.5g of extract was added and shaken to dissolve. A dirty precipitate observed indicated the presence of tannin. (b) About 0.5g of the extract was boiled in 10ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and the solution observed for brownish green or a blue-black colouration.

Test for Flavonoids

Two methods were used to test for flavonoids: (a) A portion of the extract was heated with 10ml of ethyl acetate over a steam bath for 3 minutes, the mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow colouration indicated the presence of flavonoids. (b) Dilute ammonia (5ml) was added to a portion of an aqueous filtrate of the extract. Then, concentrated sulphuric acid (1ml) was added. A yellow colouration indicated the presence of flavonoids.

Test for Alkaloids

Extracts were dissolved individually in dilute HCl and filtered. (a) filtrates were treated with Mayer's reagent (potassium mercuric iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids. (b) Filtrate was treated with Dragendorff's reagent (solution of potassium bismuth iodide). Formation of red precipitate indicates the presence of alkaloid. Filtrate was treated with Hager's reagent (saturated picric acid solution). Presence of alkaloid is confirmed by the formation of yellow coloured precipitate.

Test for Phytate

5ml of the sample was mixed, cured for 2hours and filtered. Aliquots of 2500mL of the filtrate in a conical flask was added to 5mL of 0.30% ammonium thiocyanate, the mixture was titrated with standard iron (III) chloride solution to a persistent brownish-yellow coloration that persisted for 4 minutes which indicates the presence of phytates.

Test for Cyanogenic Glycosides

One (1) ml of aqueous extract was mixed with 1ml of 20% solution of 3,5 dinitrosalic acid in methanol and 1m of a 5% aqueous NaOH was added. An immediate bright orange color was observed, an indication of the presence of cardenolides in the extract. This color fades gradually through reddish brown to brownish yellow. And it indicated the presence of glycosides. It was heated in boiling water to get brick red coloration.

Statistical analysis

All the data were subjected to analysis of variance (ANOVA) using Statistical Package for Social Sciences version 17.0 for windows, SPSS Inc. Means were separated using Duncan Multiple Range Test where significant.

Results

Proximate Compositions of the Vegetative Plant Parts

The results of the proximate composition of the vegetative parts of *Asystasia gangetica* showed that the leaves had the highest amount of carbohydrates (12.92±0.02), protein (18.4±0.44), lipid (4.95±0.2), and ash (12.8±0.2) than the roots of the plant. The roots of the plant contained the highest amount of Moisture (76.7±0.2) than the leaves, as shown in Table 4.1 below.

Table 1. Proximate Composition of the Leaves and Roots of *Asystasia gangetica*

Plant extracts	Carbohydrate CHO (%)	Protein (%)	Moisture (%)	Lipid (%)	Ash (%)	Fibre (%)
<i>Asystasia gangetica</i> leaves	12.92±0.02	18.4±0.44	3.9±0.4	4.95±0.2	12.8±0.2	46.61±0.22
<i>Asystasia gangetica</i> Root	9.2±0.06	8.97±0.22	76.7±0.2	1.25±0.05	7.5±0.1	3.53±0.04

Values represent mean ± standard deviation of triplicate sample of variance

Phytochemical Screening of Plant Extracts

The results of phytochemical investigation of the leaves and roots of *A. gangetica* is shown in Tables 2 & 3.

Qualitative Phytochemical Screening

The plant in this research was investigated for the following phytochemicals: Tannin, saponin, phytate, flavonoid, alkaloid and cyanogenic glycoside. The results indicated the presence of all the phytochemicals in the leaves and roots, of the plant (Table 2). These findings suggest that this plant is a potential source of natural antioxidants that could serve great importance as therapeutic, anti-inflammatory, anti-analgesic and anti-hyperlipidemic agent.

Table 2. Qualitative Phytochemical Screening of the Plant Species Studied

S/N	Parameters	Leaf	Root
1	Tannin	+	+
2	Saponin	+	+
3	Phytate	+	+
4	Flavonoid	+	+
5	Alkaloid	+	+
6	Cyanogenic glycoside	+	+

Quantitative Phytochemical Determination

Quantitative phytochemical analysis of the leaves and roots of *A. gangetica* showed that the leaves contain higher quantity of tannin (148.75 ± 0.00), saponin (5.61 ± 0.01), flavonoid (13.18 ± 0.00), alkaloid (15.93 ± 0.01) and cyanogenic glycoside than the roots where the quantities were 64.76 ± 0.01 , 1.8 ± 0.00 , 2.55 ± 0.00 , 9.25 ± 0.01 and 100 ± 0.00 respectively. The root only had higher quantity of phytate which was 19.43 ± 0.01 , compared to that of the leaves which was 11.67 ± 0.01 . Generally, there is a significant difference between the phytochemical compositions of both parts of *A. gangetica* as the leaf recorded more phytochemical concentration than the root at $P \leq 0.05$, as shown in Table 3 below:

Table 3. Quantitative Phytochemical Compositions of Leaves and Roots of *A. gangetica*

PARAMETERS	LEAF	ROOT
Tannin	148.75 ± 0.00	64.76 ± 0.01
Saponin	5.61 ± 0.01	1.8 ± 0.00
Phytate	11.67 ± 0.01	19.43 ± 0.01
Flavonoid	13.18 ± 0.00	2.55 ± 0.00
Akaloid	15.93 ± 0.01	9.25 ± 0.01
Cyanogenic Glycoside	120 ± 0.00	100 ± 0.00

Values are means \pm SD for 3 determinations.

Discussion

Studies have shown that dietary fiber, polyunsaturated fatty acids (PUFA), proteins, amino acids and other bioactive compounds are beneficial nutrient components (Andlauer and Fürst, 2002). The proximate composition in this study showed that *A. gangetica* leaves and roots contained protein, fiber, ash, lipids, moisture as well as carbohydrate in varying quantity as shown in Table 1. The result suggests that the leaves and roots of this plant could serve as better sources of dietary carbohydrate, protein and lipids. They add to the calorific value of food and possess odour and flavour carrying ability thereby enhancing the palatability of food. Though, data on proximate analysis of this plant is scarce.

The leaves of the plant studied contained more carbohydrate (12.92 ± 0.02) than the root. Carbohydrates are a major class of naturally occurring compounds; they are the sugars, starch and fibers found in vegetables, grains, fruits etc. Though often found in most diets, carbohydrate is a major source of energy to the human body when broken down as glucose [20]. Glucose is the most important carbohydrate in the human body [21]. Glucose is formed from the hydrolysis of complex carbohydrates such as starch, dextrin. Glucose is found in the blood and provides energy to the body. It can also be formed from the breakdown of glycogen into the body. Fructose is a reducing sugar found in fruits and in honey. It can be obtained in the body by action of sucrase on sucrose. Galactose is also a form of sugar and all these carbohydrates functioning for energy synthesis in the human body [22], also play an important role in the structure and function of vital body organs and nerve cells.

The leaves of *A. gangetica* had the highest composition of protein (18.4 ± 0.44) when compared to the roots. Proteins are essential nutrients for the human body; they are one of the building blocks of body tissue and serve as a source of fuel in building muscle mass. They are commonly found in animal products especially milk and eggs and also present in some plant sources such as legumes and nuts [23]. The process of repairing and growing muscles in the body is heavily dependent on proteins and amino acids. Thus, the amount of protein ingested by diet and the total amount of amino acids in the body at any one time is also crucial to muscle hypertrophy [24]. When carbohydrate and fats are not meeting the body's energy needs, protein and amino acids will be used for energy in their stead. One way this can happen is by degrading muscle protein into alanine which is converted to pyruvate in the liver. The pyruvate can be used in gluconeogenesis to produce glucose that fuels aerobic and anaerobic metabolism.

The roots of the plant contained the highest amount of Moisture (76.7 ± 0.2) than the leaves. Moisture is the presence of a liquid especially water often in trace amounts and the moisture content of any plant is the quantity of water that plant possesses and this can be used in a wide range of scientific areas. Due to the water content found in this plant, it shows it can survive and thrive mostly in waterlogged areas and may die off if found where it isn't getting enough water [25]. Moisture content can be measured by a number of methods which include distillation, dielectric method, hydrometer, infrared spectroscopy, refractometer, chemical analysis and oven drying. Oven drying is when a food sample is weighed before and after it is dried in an Oven. The change in weight indicates the level of moisture that was contained in the sample. However, due to the lengthy process of drying out which can take up to 18 hours, it is often used for plant samples [26]. The analysis of moisture content is essential to the food

Industry to control the quality of food, as well as the shelf life, in addition to helping food manufacturing Companies adhere to legal and labelling requirements [27].

The leaves of *A. gangetica* have more composition of lipid (4.95 ± 0.2) than the root. Lipids are substances which are insoluble in water but soluble in alcohol, ether and chloroform. Lipids serve as energy-storage depots and also provide the human body with thermal insulation. Lipids are an important component of the living cells because in cold conditions only lipids can serve the function of keeping the body warm thereby aiding the body from getting attacked by the cold and boosting the immune system in the process [28]. Cholesterol is the main sterol component found in nature. Sterols can also be found in vegetable oils and plant products in general but only in trace amounts. Lipids make the absorption of vitamin A, C, B, and E easier in the human body. There are three types of plant sterols including: stigma sterols - is a white solid plant sterol widely distributed and it's major function is to maintain the structure and physiology of cell membrane. Pytoecdysterol (ecdysone) - are sterols found in different plant groups via Pteridophytes, Gymnosperms, and Angiosperms and these sterols provide protection by deterring insects. It is also called insect moulting hormone. Sterol glycosides - includes sterolene, saponins and cardiac glycosides, they are in small quantities in higher plants. Saponins isolated from any plant are toxic to animals and cause haemolysis of red blood cells in low concentration.

The leaves have also been observed to contain more ash (12.8 ± 0.2) than the root of the plant studied. Ash is the solid remnants of fire. Some ashes contain natural compounds that make soil fertile. Consuming ash gourd improves the digestion and promotes a healthy body weight, as well as helping the same body to lose weight easily. When *A. gangetica* is burnt in a farmland, after the ashes are cooled, they can serve as manure to the soil and this would make other microorganisms and crops found in that area to grow massively [29-31]. Cereal grain - based balanced diets requires the inclusion of Ca, especially in diets for laying hens [32]. Ash as a source of Ca has been included in such diets by replacing limestone [33]. With the dominant presence of Ca in ash, it can be assumed that a large proportion of trace elements in wood ash would be present as carbonates and bicarbonates as suggested by [34].

The leaves of the plant have a valuable content of fibre (46.61 ± 0.22) when compared to the roots which contain 3.53 ± 0.04 . Fibre also called roughages is the part of the plant based food that the body cannot break down. The intake of fibre is associated with a low risk of heart disease, cholesterol, hypertension, stroke, type 2 diabetes, breast cancer as well as bowel cancer. A diet rich in fibre can help digestion and prevent constipation [35]. This plant is therefore recommended for people suffering from these problems mentioned above including other lethal ailments and diseases. Some insoluble fibres, such as cellulose, lignin, and hemicellulose do not dissolve in water and are found in foods such as wheat bran, whole grains, and vegetables, they absorb water and increase the intestinal bulk, which helps the intestine function properly. Soluble fibres, such as gum and pectin dissolve in water and are found in beans, oats, barley, some fruits, and vegetables and may play a role in lowering blood cholesterol and in regulating the body's use of sugar. Plant foods are the only source of dietary fibre and provide both soluble and insoluble fibres [1]. To further prove the presence of fibre in this plant during the experimental process, it took the stem of the plant more time to be grounded to a fine powder than the other vegetative parts.

Many plants contain powerful phytochemical substances that when taken in form of foods and herbs improve our health considerably. Phytochemicals protect us against many diseases. Results of the phytochemical screening of *A. gangetica* ethanolic leaf and root extract showed the presence of flavonoids, saponins, tannins, alkaloids, cyanogenic glycosides and phytate. These phytochemicals exhibit various pharmacological and biochemical actions when ingested by animals and humans. Plants used in the treatment of diseases are said to contain this phytochemicals with biological activities some of which are responsible for the characteristic odor, pungent scents and plant colour, while others give the particular plant its culinary, medicinal or poisonous abilities [36].

The result of phytochemical compositions presented in Table 3, showed that both the leaves and roots of *A. gangetica* possessed same phytochemical parameters including tannin, saponin, phytate, flavonoid, alkaloid and cyanogenic glycoside. However, the leaf of *A. gangetica* recorded highest contents for tannin, saponin, flavonoid, alkaloid and cyanogenic glycoside, while the highest concentration of phytate was recorded in the root of *A. gangetica*. The result from analysis of variance further showed that there is significant difference between the phytochemical contents of the leaves and the roots of *A. gangetica* as the F. calculated (8.12428) was higher than the F. critical (4.387374) at $p \leq 0.05$.

Several studies have also implicated the availability of these same phytochemicals in other plants as well as *A. gangetica* [37, 38]. The findings of the present study agree with the report of [39] as they reported same phytochemicals in *A. gangetica*. [40] also assessed the phytochemical contents of *A. gangetica* leaves. However, they reported lower values of tannin, saponin and cyanogenic glycoside compared to their equivalents evaluated in the current study. [41] also implicated the occurrence of same phytochemicals in *A. gangetica*.

The economic importance of phytochemicals in the society cannot be overlooked as they have been reported to portray useful pharmaceutical and medicinal uses [42]. [15] reported on the medicinal value of *A. gangetica* as they implicated the use of its leaf for the treatment of asthma and pile. Literatures have also shown the successful use of *A. gangetica* to control of pathogenic bacteria, fungi and worms [12, 41].

Tannins maybe employed medicinally in anti-diarrheal, haemostatic, and antihemorrhoidal compounds. The anti-inflammatory effects of tannins help control all indications of gastritis, esophagitis, enteritis and irritating bowel disorders [43]. Tannins have been used for immediate relief of sore throats, diarrhoea, dysentery, haemorrhaging, fatigues, skin ulcers. They have been also reported to have anti-viral, antibacterial and antiparasitic effects [44].

Saponins promote cardiovascular health due to the ability to lower cholesterol and body fat levels. Due to their hypoglycemic properties, they keep our blood sugar levels within normal limits and prevent insulin spikes. Saponins exhibit antimicrobial properties, guarding our body against fungi, bacteria and viruses. They act as antioxidants and scavenge oxidation stress; that's these compounds are used in some vaccines. It has been studied to induce cancer cell death and improve immune function [45].

Flavonoids are shown to exert beneficial effects in a multiple of disease states, including cancer, cardiovascular disease, and neurodegenerative disorders. Many of the biological actions of flavonoids are due to their antioxidant properties [46].

Phytate or phytic acid is an antioxidant. Antioxidants help to remove free radicals from cells in the body [47]. Phytic acid has this potential for treating multiple cancers; it was found to be anti-cancer against bone, prostate, ovarian, breast, liver, colorectal, skin cancer, etc, by suppressing the expression of matrix metalloproteinases (MMPs) and telomerase. It is anti-inflammatory and also anti-nutritional [48].

Alkaloids are usually odourless, colourless, crystalline, non-volatile, bitter solids, few are coloured. Alkaloids exhibit a wide range of pharmacological activities, they can be used as; anticancers, anti-asthmatics, myotics, antitussives, expectorants, anti-hypertensives, antiparasitics, etc.

Cyanogenic glycosides are based upon cyanide, a very deadly poison, but in small doses, they can serve as a muscle relaxant, it can also be used to suppress and soothe dry coughs [49].

Conclusion

This present study elucidated the importance of the nutritional composition and the quantitative and qualitative phytochemicals constituents inherent in *A. gangetica* leaves and roots and their utilization should be strongly recommended for good health, though the quantities of the nutritional and phytochemical components were generally higher in the leaves than the roots, except phytate, which leads to recommendation of the use of the leaves more than the roots in culinary and herbal medicinal activities. The presence of these phytochemicals justifies the use of the plant in curing certain diseases. The presence of flavonoids which are hydroxylated phenolics in the plant might be responsible for the therapeutic effectiveness against a wide array of microorganisms, probably due to their ability to complex with extracellular and soluble proteins and to complex with the bacterial cell wall. Furthermore, the high protein and fibre contents present in the *A. gangetica* leaves and roots indicated that the plant could serve as an important material for both human and animal consumption.

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