

Identification, quantification of phytochemicals and Elemental Analysis from ethanolic leaf extract of *Andrographis paniculata*

Abstract

Andrographis paniculata a nutritive which has been acclaimed medicinally for treatment of diabetics, hypertension, diarrhea and other diseases ethnomedically was screened for its pharmaceutically active ingredients and elemental compositions. The result of qualitative phytochemical showed the presence of alkaloid, phenol, tannin, flavonoid, terpenoid, saponin, steroid and cardiac glycoside which have many pharmacological properties and therapeutic activities, the quantitative phytochemical result revealed the concentration of the phytochemicals as Dihydrocytisine (1.89), ammodendrine (4.3), spartein (3.94), phytate (4.29), hydroxylupanine (7.2), kaempferol (2.68), sapogenin (8.72), tannin (2.41), ephedrine (7.16), ribalinidine (5.94), anthocyanin (12.11), flavones (10.59), flavonones (9.19), aphyllidine (11.67), proanthocyanidin (5.29), isolupanine (1.05), cyanogenic glycoside (5.65) and narigenin(7.31) in mg/kg. The FTIR result also revealed that the plant has amine, amide, ketone, phosphine, alkene and carboxylic acid functional groups confirming the presence of alkaloid, flavonoid, tannins and saponins. The X-ray fluorescence result of the elemental analysis showed that the leaf has 23 out of the 27 elements analyzed in different concentrations, which was within the accepted concentration required with calcium (64mg/kg), silicon (2.7mg/kg) and sulphur (1.4mg/kg) been the highest concentration in descending order. While Arsenic, silver, niobium and titanium were absent which are heavy metal. The elements and phytochemicals present possess pharmacological activities which confirm the ethnomedical claim of the local populace.

Keywords: *Andrographis paniculata*; mineral elemental analysis; phytochemicals; gas chromatogram-flame ion detector (GC-FID), X-ray fluorescence, FTIR.

Introduction

The use of medicinal plants and plant derived drugs as alternative medicine and food supplements to cure various ailments have increased extensively currently, though it have been in use for centuries.^[1] In many developing countries, it is estimated that about two third of the population relies heavily on traditional practises and medicinal plants to meet primary healthcare needs^[2] A medicinal plant is any plant, which in one or more of its organs contains active ingredients which can be used for therapeutic purposes or contain foundation compounds that can be used for the synthesis of useful drugs. They have invariably been rich sources of new drugs and many drugs in use today were either obtained from plants or developed using their chemical structure as templates^[3]. In modern times plants play significant role and belief is that

they have vast potential for treatment of diseases and they are supposed to be safe in use, effective, and simple with no or lesser side effects. *Andrographis paniculata* has been identified as a multipurpose medicinal plant belonging to family *Acanthaceae* ^[4]. The whole parts of the plant are bitter in taste and due to its bitterness property, it is commonly known as “King of Bitter”. As a result of the numerous problems associated with orthodox drugs, many plant species are now being revalued by researchers based on variation in plant species and their therapeutic chemical principles. Therefore, the need to do a thorough scientific based research on some species with a view to update the current state of knowledge is imperative.

Ethnomedical uses of the plant

The plant is acclaimed to be highly demanded in the pharmaceutical industries, nutritional values, food supplements, food preservatives as it is said to promote growth and health of livestock. It has been reported that the plant is beneficial for removal of toxins from the body, lowering body temperature, prevent respiratory infections and also act as antidote against poison ^[5]. Traditionally in Asian countries the plant is used as immune booster to treat fever, herpes, sore throat, gastrointestinal and respiratory tract infections as reported by Wangboonskul et al ^[6]. In Asia and Europe the whole plant, leaves and roots were used for folklore remedy ^[7]. The WHO reported that the herb *Andrographis paniculata* is widely used in Asia for cure of fever, herpes, **diarrhea**, inflammation, respiratory infection, throat sour, and various other infections ^[8]. According to Unani system of medicine, the plant is regarded as emollient, aperient, anti-inflammatory, astringent, carminative, diuretic, anthelmintic, gastric and liver tonic ^[9]. The whole plant has been used for several applications such as anti-dote for snake-bite and poisonous stings of some insects, and to treat dyspepsia, influenza, dysentery, malaria and respiratory infections ^[10, 11]. The leaf extract is a traditional remedy for the treatment of infectious disease, fever-causing diseases, colic pain, loss of appetite, irregular stools and **diarrhea** ^[12]. In Malaysia, a decoction of the aerial parts is used to treat common cold, hypertension, diabetes, cancer, malaria and snakebite ^[13]. In Ayurvedic medicinal system, tribals of Tamilnadu, India use this herb for a variety of ailments like dysmenorrhoea, leucorrhoea, prenatal and postnatal care, complicated diseases **such malaria**, jaundice, **gonorrhoea** and general ailments like wounds, cuts, boils and skin diseases ^[13].

Taxonomy of the plant

Kingdom: *Plantae*, Plant
Subkingdom: *Tracheobionta*, Vascular
Superdivision: *Spermatophyta*, Seed plants
Division: *Angiosperms*
Class: *Dicotyledonae*
Subclass: *Gamopetalae*
Series: *Bicarpellatae*
Order: *Personales*
Tribe: *Justicieae*
Family: *Acanthaceae*
Genus: *Andrographis*
Species: *A. paniculata*^[14]



Fig. 1 : Morphology of plant

Material and Methods:

All the materials for the analysis were of analytical grade

Acetic acid (BDH), Amyl alcohol (JHD), Chloroform (BDH 99%) Corona mechanical Grinder 2013 model, Hydrochloric acid (BDH m.w 36.46), Methanol (BDH), Sulphuric acid (A.R Fisons S.G 1.84 m.w 98.08)

Instrument

M910 Gas Chromatography equipped with a flame ionization detector (GC-FID)

Agilent 8400 FTIR machine for functional group determination

EDX3600B X-ray fluorescence (EDXRF) spectrophotometer (Precision- 0.01–0.05% deviation; Detection limit- 0.0001–99.9999%) by Sky ray Instrument

Methods

Plant collection and identification

Fresh leaves of *Andrographis paniculata* were collected from a land in Akwakuma Uratta Owerri North Local Government of Imo State Nigeria and were identified by Prof F.N Mbagwu of Plant Science Department Imo State University.

Preparation of the sample for analysis

Andrographis paniculata leaves and stem bark samples were washed with water and then dried for 2 weeks at room temperature. The plant materials following dryness was powdered with new mechanical grinder. The powdered leaves and stem barks were weighed (2.2 kg) and stored in amber coloured Winchester bottle. The powdered *Andrographis paniculata* leaves and stem barks (500 g) were percolated with 1000 ml of redistilled ethanol (99 %) for 24 hours. The extracts were filtered and concentrated in a water bath at 55 °C.

Qualitative phytochemical screening

Qualitative Phytochemical analysis of the plant extracts was carried out in order to confirm the presence of phytochemicals as shown below.

Test for Alkaloid

Preparation of Wagner's Reagent:

Wagner's Reagent: 1.3g of iodine crystals and 2.0g of KI were dissolved in 100ml volumetric flask made up to the mark with distilled water. Reddish brown colour on the addition of the reagent to the extract indicated the presence of alkaloid ^[15].

Test for Saponins:

0.5g. of dried plant powdered material was extracted with 5ml of 50% aqueous methanol solution. Extracted material was transferred into a test tube and was well agitated. Formation of persistent foam at the surface was taken as an indication for saponin ^[16].

Test for Tannin:

0.1g of plant material was measured into a test tube and 3ml of butanol-HCl reagent (95ml of n-butanol and 5ml of concentrated HCl) were added to it. The test tube was plugged with cotton and was heated on boiling water bath for an hour. Appearance of pink colour indicated the presence of tannin ^[16].

Test for Phenol

Ferric chloride test: 2ml of plant extract were measured out and 2ml of distilled water added followed by addition of 10 % FeCl₃ solution. Bluish black colour indicates the presence of phenol ^[17].

Test for Terpenoids

Sulphuric acid Test: Crude plant extract was dissolved in 3ml of chloroform. This was then evaporated to dryness and 2ml of conc. H₂SO₄ was added and heated for about 3minutes. A grayish colour indicated the presence of terpenoids ^[17].

Test for Steroids

Sulphuric acid Test: To the plant extract 2 ml of chloroform was added. 2 ml of conc. H₂SO₄ was added by the sides of the test tube and appearance of red color at lower chloroform layer indicated the presence of Steroid. ^[17].

Test for Flavonoid

4 ml of extract were taken and about 2ml of 50% methanol added. The solution was warmed and 1g of magnesium metal was added. This was followed by addition of 5 to 6 drops of concentrated hydrochloric acid. Red coloration confirmed the presence of flavonoids ^[17].

Fourier transform infrared spectrophotometric analysis

The FTIR spectroscopic analysis was carried out using FTIR-8400s Fourier Transform infrared Spectrophotometer by Springboard laboratory Awka. This helped in determination of functional groups present in the sample.

The results of the functional group present in the sample were shown in appropriate tables.

Gas chromatogram-flame ionization detector

The identification of phytochemicals in the crude extract was performed on a Buck M910 Gas Chromatography equipped with a flame ionization detector (GC-FID) using ARESTEK 15m MXT1 column (15m×250µm×0.15µm). The injector temperature was 280°C with split less injection of 2 µL of sample and a linear velocity of 30 cms⁻¹. Helium 5.0 Pa.s was the carrier gas with a flow rate of 40mL/min. The oven operated initially at 80°C and then heated to 330 °C at a rate of 5 °C/min, and kept at this temperature for 5 min. The detector was operated at a temperature of 320 °C. Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals ^[18].

Elemental Analysis

The leaves for elemental analysis were oven dried at 100 °C for 4 h and ground into powder using an electric grinder. The ground leaves were sieved to 30 mesh particle size using American Society for Testing and Materials (ASTM) standard sieves. The powdered leaf was then stored in moisture free and air tight plastic container. The mineral element composition of the leaf was determined using EDX3600B X-ray fluorescence (EDXRF) spectrophotometer (Precision- 0.01–0.05% deviation; Detection limit- 0.0001–99.9999%) by Sky ray Instrument. The energy dispersive X-ray spectra were obtained by pulverizing and pelletizing the powdered leaf. The fluorescence spectrophotometer was calibrated using pure silver standard and the working curve for the sample selected before the sample analysis ^[18]. The result recorded in the appropriate table

Results and Discussion

Table 1: Result of Phytochemical Screening of the leaf of *Andrographis paniculata*

Phytochemicals	Result
Alkaloid	+
Flavonoid	+
Saponin	+
Terpenoid	+
Tannin	+
Steroid	+
Phenol	+

+ = Present

Phytochemical screening of the ethanolic crude leaf extract of *Andrographis paniculata* was done and the result revealed the presence of Alkaloids, Flavonoids, Saponins, Terpenoid, Phenol, Tannins, and Steroid. These compounds are known to show curative activities against several pathogen and therefore could explain its used traditionally for the treatment of wide array of illnesses. The phytochemicals present in the plants may be responsible for preventing disease and promoting health. Findings by some researchers revealed that phytochemicals may reduce the risk of coronary heart disease by preventing the oxidation of low-density lipoprotein (LDL) cholesterol, reducing the synthesis or absorption of cholesterol, normalizing blood pressure and clotting, and improving arterial elasticity. Phytochemicals may detoxify substances that cause cancer^[19]. The phytochemical result corresponded to the ethomedical use of the plant. The reported use of the plant leaf ethnomedically in treatment of pains, fever, inflammation, respiratory infection, throat sour, diarrhea, management of diabetes and hypertension may be due to the presence of alkaloid, while the reported ability of the leaf to stop dysentery, diarrhea and duodenal tumour may be as a result of the presence of tannins. The flavonoid in it may serve as

antioxidant and free radical scavenger. They reported finding that the leaf is used to neutralize the acidity of inflammations may be due to the presence of (OH) bond in their benzene ring ^[20] which may also lower cholesterol level in man ^[21]. Varied biological activities of phenolic acids have been reported which include increase bile secretion, reduces blood cholesterol and lipid levels and antimicrobial activity against some strains of bacteria such as *Staphylococcus aureus*. Phenolics acid possesses diverse biological activities, for instance, antiulcer, anti-inflammatory, antioxidant, cytotoxic and antitumor, antispasmodic, and antidepressant activities^[22]. The presence of alkaloid in extracts was reported by previous researchers to inhibit the growth of *Staphylococcus aureus*, they are used as antimycotics, and in the treatment of stomach pains ^[23] Alkaloids have many pharmacological activities including antihypertensive effects (many indole alkaloids), antiarrhythmic effect (quinidine, sparteine), antimalarial activity (quinine), and anticancer actions (dimeric indoles, vincristine, vinblastine). Flavonoids may be responsible for diuretic and antibacterial activity, since they are antioxidants. They may also help in healing of wounds and in treatment of skin diseases due to their ability to neutralize the acidity of wounds and inflammations. They are also used in treatment of diarrhea.^[20] Flavonoids, a subclass of poly phenols, are groups of phytochemicals that are among the most potent and abundant antioxidants in our diet and also possesses various activities such as anti-inflammatory, anti-cancer etc.^[24]. Tannin extracts have been reported to be anti-inflammatory, control gastritis and irritating bowel disorder, they may also contribute to antimicrobial power which heals wounds and stop bleeding ^[23]. Tannin also ensures the inhibition of organism by coagulating their micro protoplasm^[20]. Tannin-containing plant extracts are used as astringents, against diarrhea, as diuretics, against stomach and duodenal tumors, and as anti-inflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals.^[25]

Saponin detected in the plant has been found to be an antibacterial and antimycotic substances on cell wall of many organisms.^[26] Saponin mixtures present in plants and plant products possess diverse biological effects when present in the animal body. Extensive research has been carried out into the membrane-permeabilising, immunostimulant, hypocholesterolaemic and anticarcinogenic properties of saponins and they have also been found to significantly affect growth, feed intake and reproduction in animals. These structurally diverse compounds have also been observed to kill protozoans and molluscs, to be antioxidants, to impair the digestion of

protein and the uptake of vitamins and minerals in the gut, to cause hypoglycaemia, and to act as antifungal and antiviral. [27]

Terpenoids have medicinal properties such as anti-carcinogenic (e.g. perilla alcohol), antimalarial (e.g. artemisinin), anti-ulcer, hepaticidal, antimicrobial or diuretic (e.g. glycyrrhizin) activity and the sesquiterpenoid antimalarial drug artemisinin and the diterpenoid anticancer drug taxol. [28]

Table 2: Result of the elemental composition analysis of Leaf of *Andrographis paniculata*

Element	Content (mg/kg)
Magnesium (Mg)	0.1
Aluminum (Al)	0.1
Silicon (Si)	2.7
Phosphorus (P)	0.4
Sulphur (S)	1.4
Potassium (K)	0.01
Calcium (Ca)	64.2
Titanium (Ti)	0.00
Vanadium (V)	0.01
Chromium (Cr)	0.00
Manganese (Mn)	0.01
Cobalt (Co)	0.01
Iron (Fe)	0.95
Nickel (Ni)	0.01
Copper (Cu)	0.02

Zinc (Zn)	0.04
Arsenic (As)	0.00
Lead (Pb)	0.02
Tungsten (W)	0.01
Gold (Au)	0.02
Silver (Ag)	0.00
Rubidium Rb	0.01
Niobium Nb	0.00
molybdenum (Mo)	0.2
Cadmium (Cd)	0.01
Tin (Sn)	0.45
Antimony (Sb)	0.43

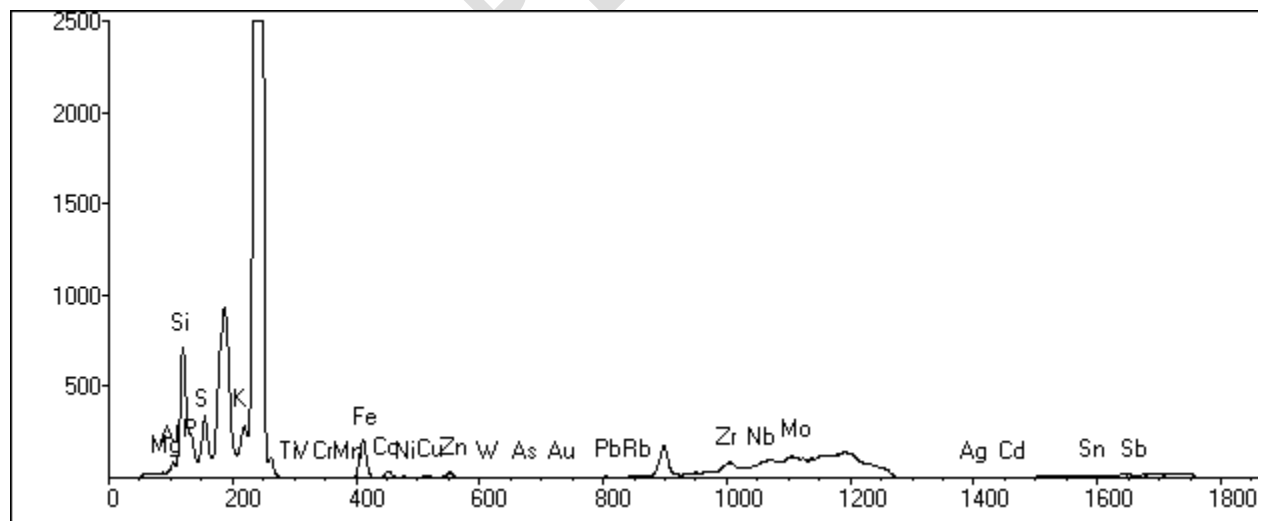


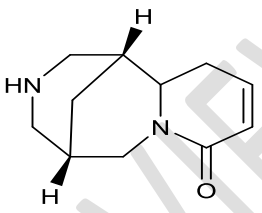
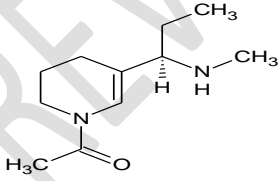
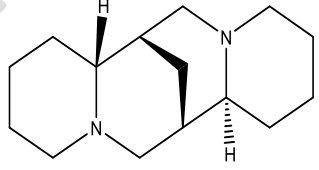
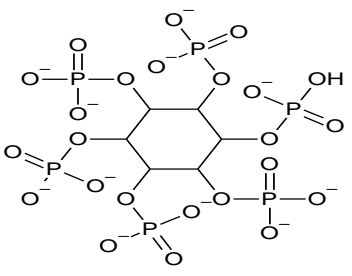
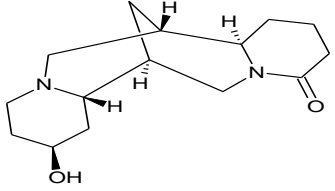
Fig .2 Elemental composition analysis of Leaf of *Andrographis paniculata*

Reseachers have reviewed the importance of minerals in animals and man. Appreciable amounts of Calcium, Silicon, Sulphur, Iron, Selenium, Antimony, Molybdenum and **potassium** and

Silicon were found in the analyzed plant in descending order. Calcium is a major constituent of bones and teeth and takes part in the regulation of nerve and muscle function. During the coagulation of blood, calcium activates the conversion of prothrombin to thrombin. It activates large number of enzymes and is also required for membrane permeability ^[29]. This mineral had the highest concentration in the plant. Silicon and Sulphur **was** the second and third highest sulphur is present in cystine, cysteine and methionine which are amino acids. It is found in connective tissues, skin, hair and nails. The molecules of thiamine, biotin (member of vitamin B complex) and coenzyme A also contain sulphur. Potassium ions are very important in intracellular fluids and play a key role in acid-base balance, osmotic pressure regulation, transfer of nerve impulse, contraction of cardiac muscles, cell membrane function and in glycogenesis. It also helps in the transfer of phosphate from ATP to pyruvic acid. Its concentration in the plant was not high. Silicon is the second highest in the plant it is an important component of certain mucopolysaccharides, hyaluronic acid and chondroitin-4-sulphate, found in connective tissues. It acts as a biological cross-linking agent and plays a role in the structure and resiliency of connective tissues. It is also important in bone calcification. Iron, phosphorus, zinc and molybdenum were found in moderate amounts in plant. Iron is a component of blood haemoglobin, erythrocytes and plasma. Its functions include oxygen transportation, cellular respiration and an essential component of enzymes involved in biological oxidation such as cytochromes. It is a cofactor for a number of enzymes and helps in the proper myelination of spinal cord and white matter of cerebellar folds in the brain. It also plays a role in neurotransmitter synthesis. Phosphorus functions as a constituent of bones, teeth, adenosine triphosphate (ATP), phosphorylated metabolic intermediates and nucleic acids. It is also involved in the synthesis of phospholipids and phosphoproteins. Zinc is found in many enzymes which are involved in cell replication and micronutrient metabolism. Molybdenum is a component of several metalloenzymes and is involved in the utilization of iron as well as in electron transport during cellular metabolism. Trace amounts of copper, nickel, aluminium, cobalt, vanadium and manganese were also found in plant. Copper is an essential micro-nutrient essential in the formation of bones, myelin sheaths in the nervous systems and helps in the addition of iron to the hemoglobin. It also assists in the transfer of iron from tissues to the plasma as well as its absorption in the gastrointestinal tract. Nickel is an essential element in animals which has been associated with the control of prolactin, maintenance of membrane structure and

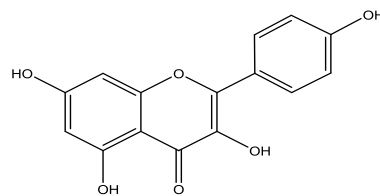
nucleic acid metabolism. Manganese helps in the synthesis of proteoglycans in cartilage and is involved in the biosynthesis of connective tissues, formation of urea and metabolism of pyruvate [18].

Table 3: Result of Quantitative phytochemical analysis

Phytochemicals	Concentration(mg/kg)	Structure
Dihydrocytisine	1.8911	
Ammodendrine	4.2628	
Sparteine	3.9353	
Phytate	4.2911	
Hydroxylupanine	7.2087	

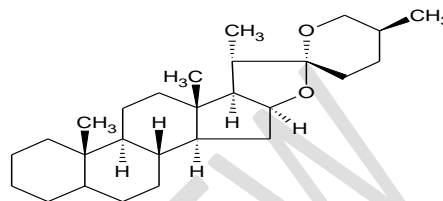
Kaempferol

2.6852



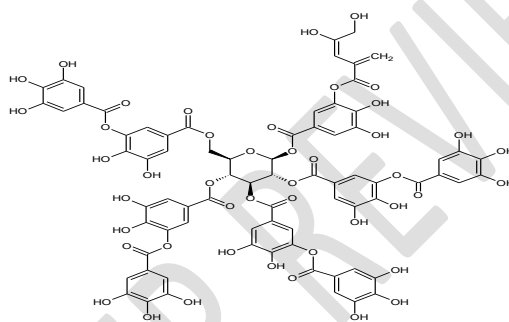
Sapogenin

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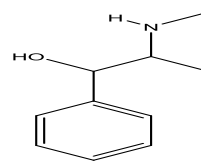
Tannin

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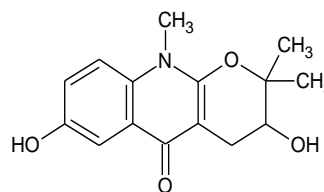
Epihedrine

7.1555



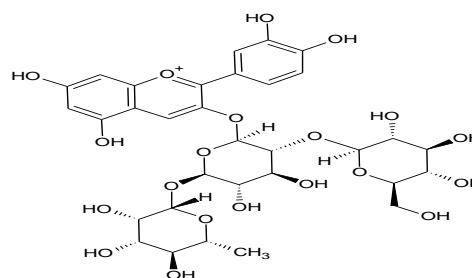
Ribalinidine

5.9387



Anthocyanin

12.1123



Narigenin

7.3064

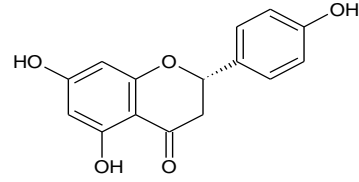


Fig .3 Chemical structure of different chemicals

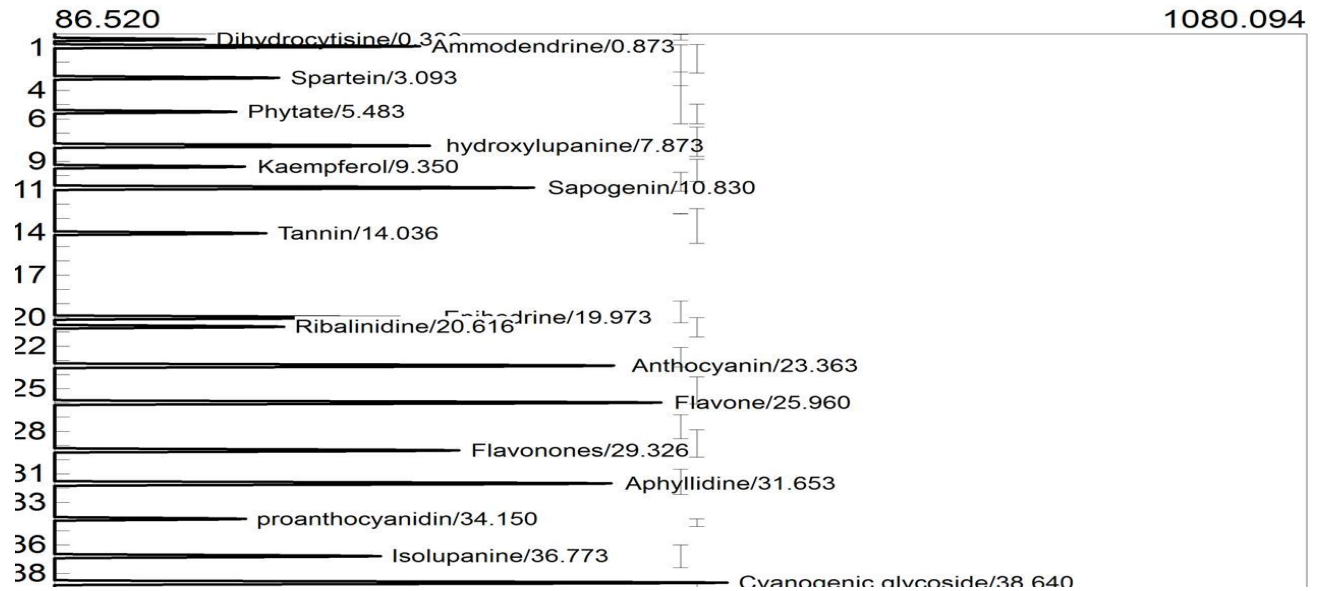
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Temperature program:

Init temp	Hold	Ramp	Final temp
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Component	Retention	Area	Height	External	Units
Dihydrocytisine	0.386	2631.0434	206.270	1.8911	ppm
Ammodendrine	0.873	4824.9767	377.473	4.2628	ppm
Sparteine	3.093	3389.3006	265.800	3.9353	ug/ml
Phytate	5.483	2954.8422	231.937	4.2911	ug/ml
hydroxylupanine	7.873	4968.2281	389.420	7.2087	ppm
Kaempferol	9.350	3057.1151	240.132	2.6852	ug/ml
Sapogenin	10.830	6003.0656	470.455	8.7178	ug/ml
Tannin	14.036	3250.6604	255.555	2.4131	
Epihedrine	19.973	4928.6840	387.165	7.1555	ug/ml
Ribalinidine	20.616	3419.6849	268.983	5.9387	ug/ml
Anthocyanin	23.363	6954.4788	536.637	12.1123	ug/ml
Flavone	25.960	7289.2832	570.180	10.5857	ug/ml
Flavonones	29.326	5273.2844	410.247	9.1896	ppm
Aphyllidine	31.653	6802.6676	532.675	11.6684	ppm
proanthocyanidin	34.150	3084.5871	241.227	5.2864	ug/ml
Isolupanine	36.773	4449.9298	348.939	1.0515	
Cyanogenic glycoside	38.640	8024.0488	624.601	5.6520	ppm
Narigenin	43.536	5031.2166	394.788	7.3064	ug/ml
		86337.0973		111.3515	

The GC-FID phytochemical analysis showed different classes of phytochemicals which include alkaloids, phenolic compounds, flavonoid, **tannin, saponin**. The alkaloid compounds present include dihydrocytisine, ammodendrine, sparteine, hydroxylupanine, epihedrine, ribalinidine, **aphyllidine, isolupanine**. Cytisine has been used as a smoking cessation treatment since 1964, and is relatively unpopular in regions outside of central and Eastern Europe. Cytisine is a partial nicotinic acetylcholine agonist with a half-life of 4.8 hours. Recent study showed that cytisine have similar efficacy to varenicline which is a smoking cessation drug. Dihydrocytisine is a cytisine with 2 hydrogen. Cytisine also known as baptitoxine or sorphorine is an alkaloid naturally derived from the *Fabaceae* family of plants including the genera Laburnum and Cytisus. Recent studies have shown it to be a more effective and significantly more affordable smoking cessation treatment than nicotine replacement therapy. Also known as baptitoxine or sorphorine ^[30]. Ammodendrine is a piperidine alkaloid that is piperidine substituted a 1-acetyl-1,4,5,6-tetrahydropyridin-3-yl group at position 2 (The 2R-stereoisomer). it has a role as a plant metabolite and a teratogenic agent. It is a piperidine alkaloid, a-N-acylpiperidine and a member

of acetamides^[31]. The pharmaceutical worth of piperidine is extensive, its derivatives are used in over twenty drug classes including anticancer agents, drugs for alzheimer's disease therapy, antibiotics, analgesic, antipsychotics, antioxidants and others.^[32] Spertine and lupanine which are Lupine alkaloids and their derivative have neurotoxic effects through their interactions with cell receptors^[33]. The concentration of these phytochemicals increased in the order isolupanine (01.0515 mg/kg) < Dihydrocytisine (1.8911 mg/kg) < Tannin (2.4131 mg/kg) < kaempferol (2.6852 mg/kg) < Spartein (Ammodendrine) < Phytate (4.2911 mg/kg) < Proanthocyanidin (5.2864 mg/kg) < Cyanogenic glycoside (5.652 mg/kg) < Ribalinidine (5.9387) < Epihedrine (7.155 mg/kg) < Hydroxylupanine (7.2087 mg/kg) < Narigenin (7.3064 mg/kg) < Sapogenin (8.717 mg/kg) < Flavonones (9.1896 mg/kg) < Flavone (10.5857 mg/kg) < Aphyllidine (11.66mg/kg) < Anthocyanin (12.11 mg/kg). Flavonones, flavone, Aphyllidine, and anthocyanin were the major phytochemicals in *Andrographis paniculata*. Alkaloids, flavonoids and Phenolic compounds were the most abundant and are perhaps the most explored natural compounds due to their potential medicinal values as demonstrated in many studies. They have been linked to antioxidant, anticancer and antimicrobial activities. Flavan-3-ol oligomers and monomers have been reported to be potent antioxidant compounds, flavanone is a flavonoid that has been linked to cardiovascular disease and cancer prevention. Steroids found in plants have shown many medicinal activities like antibacterial, anti-tumor, growth hormone regulation and antihelminthic activity. It has been reported to show anticancer activity as well as in preventing heart damage after a cardiac arrest. It also assists in the reduction of oxidative damage of the liver during ethanol intoxication^[18]. Phytates, naringenin, kaempferol, flavones, sapogenin, ribalinidine, tannins, spartein and anthocyanins were also identified in the extract. Ribalinidine and spartein are quinoline alkaloids, and compounds with this ring are known to be pharmacologically active. Ribalinidine has been reported to have radical scavenging function. Anthocyanins are flavonoid compounds found in different parts of many plants. They have been reported to show antioxidant, anti-inflammatory and anti-cancer activities. It is an important anti-malarial drug and has been recommended in many African countries as a first line and second-line treatment for severe and uncomplicated malaria respectively. Tannins are poly phenols with numerous physiological potentials which includes antibacterial, anti-inflammatory, antioxidant, antiviral, anti-diarrheal and anti-malarial activities, another phytochemical detected was phytate the salt of phytic. It has been shown to exhibit anti-inflammatory, metal chelating and antioxidant

activities. Naringenin is a hydroxyl derivative of flavanone. It can be found in the glycosidic form as naringin. Naringenin reduces cholesterol and exhibits anticancer, anti-inflammatory, antiulcer and skin protective activities ^[18]. The phytochemical composition supported the ethnomedical claims of the plant. **The used** of the plant to treat fever and pain was due to the alkaloid compositions. **The used** of the plant for treatment of **diarrhea**, ulcer, bronchitis gastrointestinal disorder, cancer and tuberculosis may be due to the **present** of the flavonoids, tannins and phenol.

Table 4: Result of FTIR analysis of *Andrographis paniculata*

Peaks(cm^{-1})	Functional group
753.46	C-H bend of Aromatics (ortho)
845.84	P-H bend of phosphines
1302.90	C-O Stretch of carboxylic acid
1617.18	N-H bend of amides
2103.36	C=C stretch of alkenyl
2840.30	C-H stretch of aldehyde (ethers)
2987.31	O-H stretch of ketones
3186.92	N-H stretch of amines
3685.91	N-H stretch of amides

From the Fourier transform infra-red spectrophotometer analysis it was observed that the leaf **contain** some compounds having functional groups of aromatics, phosphines, carboxylic acid, amides, aldehyde, ketones and amines. The absorption band at 753.46 cm^{-1} showed that the plant **contain** C-H bend of aromatics which was showed by the presence of some phytochemicals having aromatic rings. Absorption wave number of 845.84 cm^{-1} showed a bend of phosphine which was due to the presence of phytate in the plant. The strong appearance of N-H bend and stretch of amide and amines was due to the presence of alkaloids which include spartein,

ribalinidine, aphyllidine, epihedrine, ammodendrine and hydroxylupanine. 2987.3cm^{-1} represent O-H stretch of ketone which is as a result of the presence of flavonoids in the plant.

Conclusion

The results of the study have shown that *Andrographis paniculata* extracts contain some phytochemicals like saponin, flavonoid, alkaloid, tannins, phenol, terpenoid and steroids which have antimicrobial activities, confirming the plant potency as a medicinal plant. The mineral element analysis confirm that the leaf is rich in minerals and can be use as food supplement as it contain high content of micronutrients which are beneficial to plants and man. The use of the plant in the treatment of fever, herpes, sore throat, and infections in gastrointestinal and respiratory tract by the local populace can be attributed to the presences of these phytochemicals which are known for their therapeutic properties. The phytochemical result also corresponded with that of Puranik et al who also reported the presence of saponin, alkaloid and flavonoids. It may be use in managing, skin sore, birth defect, anemia, heart failure, neurological symptoms and skeleton disorder due to the presence of some mineral element like Iron, Manganese, Zinc, Potassium, Magnesium and Calcium hence supporting its ethno-medical use. This investigation has scientifically justified the use of *Andrographis paniculata* leaf in ethnomedical practice provided it would be administered within the appropriate toxicity level for human. This research conform to the findings of previous researchers supporting its ethno-medical use. The presence of these phytochemicals and mineral elements may be the reason why the plant possesses immuno-modulatory, antioxidant antiinflammatory, cytotoxic, antidiabetic, hepatoprotective, antimalarial, antihypertensive, antimicrobial and antifertility activities. This investigation has scientifically justified the use of *Andrographis paniculata* leaf in ethnomedical practice as antioxidant, anticancer, anti-inflammation, anti-venom, anti-pyretic, anti diabetic, anti-microbial, antihyperlipidemic and hepatoprotective activities, this support the use of the leaf extract as a traditional remedy for the treatment of infectious disease, fever-causing diseases, colic pain, loss of appetite, irregular stools and diarrhea^[7]. This work supported the ethnomedical use of the plant for treatment of common cold, hypertension, diabetes, cancer, malaria and snakebite^[8] by the local populace provided it would be administered within the appropriate toxicity level for human.

References

1. Joy P, Thomas J, Mathew S, Skaria BP. Medicinal plants *Tropical Horticulture*; 1998:449-632
2. Aladesanmi AJ. *Tetrapleura tetraptera* Molluscicidal Activity and Chemical Constituents. *African Journal of tradition complementary Alternative Medicine*. 2007; 4(1): 23–36
3. Ajaiyeoba E, Falade M, Ogbale O, Okpako L, Akinboye D. In vivo Anti-malaria and Cytotoxic Properties of *Annona Senegalensis* Extract. *African journal of Trad. CAM* 2006; 3(1):137 – 141
4. Mishra SK, Sangwan NS, Sangwan RS. *Andrographis paniculata* (Kalmegh): a review. *Pharmacognosy Reviews*. 2007; 1(2):283-298.
5. Coon JT, Ernst E. *Andrographis paniculata* in the Treatment of Upper Respiratory Tract Infections: A Systematic Review of Safety and Efficacy. *Planta Medica Journal* 2004; 70:293-298.
6. Gabrielian ES, Shukarian AK, Goukasova GI, Chandanian GL, Panossian AG. A double blind, placebo-controlled study of *Andrographis paniculata* fixed combination Kan Jang in the treatment of acute upper respiratory tract infections including sinusitis. *Phytomed*. 2002; 9:589-597.
7. Wangboonskul J, Daodee S, Jarukamjorn K, Sripanidkulchai BO. Pharmacokinetic study of *Andrographis paniculata* tablets in healthy Thaimale volunteers. *Thai Pharm Health Sci. J.* 2006; 1(3):209-218.
8. Jarukamjorn K, Nemoto N. Pharmacological aspects of *Andrographis paniculata* on health and its major diterpenoid constituent andrographolide. *Journal of Health Science*. 2008; 54(4):370-381.
9. World Health Organization. WHO Monographs on selected medicinal plants Geneva: AITPBS Publications and Distributors. 2002; 2:12-24
10. Chopra RN. Glossary of Indian medicinal plants. New Delhi: Council for Scientific and Industrial Research; 1980 p. 18
11. Jarukamjorn K, Kondo S, Chatuphonprasert W, Sakuma T, Kawasaki Y, Emito N. Gender-associated modulation of inducible CYP1A1 expression by *andrographolide* in mouse liver. *Eur J Pharm Sci.* 2010; 39:394–401.
12. Saxena S, Jain DC, Bhakuni RS, Sharma RP. Chemistry and pharmacology of *Andrographis* species. *Indian Drugs*. 1998; 35:458–467.
13. Okhuarobo A, Falodun JE, Erharuyi O, Imieje V, Falodun A, Langer A. Harnessing the medicinal properties of *Andrographis paniculata* for diseases ad beyond: a review of its phytochemistry ad pharmacology. *Asia pac J. Trop Disease*. 2014; 4 (3):213-222
14. Sivananthan M, Elamaran M. Medicinal and pharmacological properties of *Andrographis paniculata* *international journal of Biomolecules and Biomedicine* 2013; 3 (2): 1-12
15. Eluyode OS, Alabi OS. Preliminary Phytochemical Screening of Crude Extracts of *Commiphora africana* on Inflammation and Pain in Rodents. *Asian Journal of Medical Science*, 2007; 2(3):811-84.
16. Aisha H, Palwasha, A, Hasnain N. Detection and Estimation of Alkaloids, Saponins and Tannins in Herbs of Quetta Baluchistan *American-Eurasian Journal of Agricultural and Environmental Science* 2015; 15(6): 985-990

17. Rajesh KS, Yadav RN. Qualitative Phytochemical Analysis and Estimation of Total Phenols and Flavonoids in leaf extract of *Sarcochlamyspulcherrima wed.* *Global Journal of Bio-Science and Biotechnology* 2015; 4(1) 81-84
18. Duru C.E. Mineral and phytochemical evaluation of zea mays husk *Scientific African* 2020; 7(e00224):1-8
19. Mamta S, Jyoti S, Rajeev N, Dharmendra S, Abhishek G. Phytochemistry of Medicinal Plants. *Journal of Pharmacognosy and Phytochemistry* 2013; 1(6) : 168-182
20. Ajiwe VI, Dimonyejiaku NN, Ajiwe AC, Chinwuba AJ, Chendo, NM. Preliminary Study on the Pharmaceutical Constituents of *emiliasonchifolia* leaf, *Anachem Journal*, 2008; 2 (2): 302-309.
21. Nwokolo E, Iechukwu SN. Food and feed from legume and oil seeds, Chapman and Hill, London. 1996; 229-239
22. Ghasemzadeh A, Jaafar HZ, Rahmat A. Antioxidant activities, total Phenolics and flavonoids content in two varieties of Malaysia Young Ginger (*Zingiberofficinale Roscoe*). *Molecules*, 2010; (15): 4324-4333.
23. Ikezu UJM, Udeozo IP, Egbe DE. Phytochemical and Proximate Analysis of Black Turtle beans (*Phaseolusvulgris*) Proceedings of the 37th Annual International Conference, Workshops and Exhibition of Chemical Society of Nigeria (CSN) 2014; (2): 276-278.
24. Tapas AR, Sakarkar DM, Kakde RB. Flavonoids as Nutraceuticals: A Review. *Tropical Journal of Pharmaceutical Research*, 2008; (7): 1089-1099.
25. Dolara P, Luceri C, De Filippo C, Femia AP, Giovannelli L, Carderni G, Cecchini C, Silvi S, Orpianesi C, Cresci A. Red wine polyphenols influence carcinogenesis, intestinal microflora, oxidative damage and gene expression profiles of colonic mucosa in F344 rats. *Mutation Research*, 2005; (591): 237–246
26. Benefits of Saponins. www.medicalmarijuana.com Retrieved 20/01/2021.
27. Takechi M, Matsunami S, Nishizawa J, Uno C, Tanaka Y. Haemolytic and antifungal activities of saponins or anti-ATPase and antiviral activities of cardiac glycosides. *Planta Medica*, 1999; (65): 585–586
28. Langenheim JH, Higher plant terpenoids: A phyto-centric overview of their ecological roles. *Journal of Chemical Ecology*, 1994 (20): 1223-1280
29. Soetan KO, Olaiya CO, Oyewole OE. The importance of mineral elements for human, domestic animals and plants a review. *African journal for food science* 2010; 4 (5): 200-222
30. Cytisine <https://pubchem.ncbi.nlm.nih.gov/compound/Cytisine> Retrieved 20/07/2021
31. Ammodendrine: <https://pubchem.ncbi.nlm.nih.gov/compound/ammodendrine>. Retrieved 20/07/2021
32. Pharmacological applications of piperidine derivatives <https://encyclopedia.pub/entry/40989> Retrieved 29/05/2023
33. Trugo LC, Von Baer D, Von Baer E. Lupine encyclopedia of food sciences and nutrition 2003; 2: 3623-3629.