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RESEARCH ARTICLE

Profiling of Phytochemicals in the Leaves of *Asystasia gangetica* (L) T. Anderson using GC-MS and HPLC Analysis

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Abstract

Asystasia gangetica is a perennial herb that has naturalized in Africa and Asia, where it is use in treatment of varying ailments such as lowering of blood sugar, sexually transmitted infections, reduce swelling and ear disease. The presence of phytochemicals in this plant may be responsible for its acclaimed effects. This study aims to identify the phytoconstituents in the leaves of Asystasia gangetica. Preliminary phytochemical screening was achieved by standard methods, GC-MS and HPLC analyses were utilized to determine volatile and non-volatile compounds. Phytochemicals identified include alkaloids, tannins, steroids, saponins, flavonoids, and glycosides. GC-MS analysis identified twenty-eight compounds with Z-(13,14-Epoxy)tetradec-11-en-1-olacetate (8.72 %); Octadecane, 1-(ethenyloxy)- (8.77 %); Cycloheptano[d]imidazolidine, 1,3- (9.81 %); cis-Vaccenic acid (10.20 %); 15-Hydroxypentadecanoic acid (11.94 %) as the prominent compounds and HPLC evaluation reported the most prominent compounds such as proanthocyanidin (6.69 %), flavone (7.51 %), spartein (7.64 %), aphylidine (10.33 %) and cyanogenic glycoside (10.51 %) respectively. Identification of these compounds with documented evidence of pharmacological activities, thus validate the use of *Asystasia gangetica,* in many disease conditions.

Keywords: Profiling; A*systasia gangetica*; Phytochemicals; Chromatographic technique

Introduction

Asystasia gangetica (L) T. Anderson, family Acanthaceae, with two subspecies *Asystasia gangetica* subspecies *gangetica and Asystasia gangetica* subspecies *micrantha.* It was once known scientifically as *Justicia gangetica* and *Asystasia coromandeliana* Nees and commonly called Chinese violet and creeping foxglove [1]. *A. gangetica* is an erect or decumbent perennial herb that grows to the length of 1.0 to 3.0 m, leaves with simple blade but oppositely arranged and numerous cystoliths on the upper surface, while stipulate is absent [2]. *A. gangetica* is native to India, Malaysia and Africa but have been distributed to parts of the United State of America and Australia [3, 4, 5] Closely

related species of *A. gangetica* include *Asystasia acuminata Klotzsch, Asystasia bojeriana Nee, Asystasia coromandeliana Nees, var micrantha Nees, Asystasia floribunda Klotzsch, Asystasia intrusa (Forssk) Blume, Asystasia multiflora Klotzsch, Asystasia parvula C.B. Clark, Asystasia podostachys Klotzsch, Asystasia pubescens Klotzsch, Asystasia quarterna Nees Asystasia querimbensis Klotzsch, Asystasia scabrida Klotzsch, Asystasia subhautata Klotzsch, Asystasia travancorica Bedd* and *Asystasia variabilis* [6,7]. These species vary genetically, which may enhance their survival in different environments. They achieve this by the production of phytochemicals, which protect them from predators and harsh environmental factors [8]

Tender leaves and shoots of *A. gangetica* are eaten as fried or boiled vegetable. In African folk medicine, the leaves are used in the treatment of diabetics melitus and asthma. Juices from the leaves are used to manage swelling, rheumatism, antihelminthics, gonorrhoea and ear disease. Paste from the root is applied to manage skin allergy [9, 10, 11]. Powdered roots of the plant are used to treat snake bites and stomach aches. Decoction from the leaves is used in the treatment of epilepsy, urethral discharge, and pain. The leaves are popular in Nigeria as a treatment for asthma. In India, the sap is used as a vermifuge. Pharmacological studies have validated the analgesic and anti-inflammatory [12], antihypertensive [13], antiulcer [14], Antibacterial [15], antioxidant [16] and antiasthmatic [17] properties of *A. gangetica*The traditional uses and pharmacological potentials associated with *A. gangetica* can be ascribed to the presence of phytochemicals in the various parts of the plant. Identifying these phytochemicals could validate its claimed uses and biological activities, and also other yet-to-be-identified uses of the plant. Previous studies have identified phytochemicals in different parts of the plant, using different solvents. They include the use of GC-MS in the identification of phytochemicals in methanol root extract and benzene leaf extracts of *A. gangetica*. Also, essential oils from the aerial part, seeds and roots have been evaluated [18, 19]. Twenty phytochemicals were identified from the methanol root extract, some of which include Cervinomycin A1-trimethyl ether (antibacterial); Lycoxanthin (anti-inflammatory, antioxidant); Ungeremine (cytotoxic-anticancer); Emodin 1,8-dimethylether (anti-rheumatoid arthritis). Also, D1-Allo-cystathionine (antibacterial); N-ethyl-N-nitroguanidine; N-methyl-1-Adamantaneacetamide (analog-larvicidal) were identified from the benzene leaves extracts [18, 19]. A search for compounds identified by GC-MS and HPLC analysis of the methanol extract of *A. gangetica* has not been reported. Thus, this work intends to identify phytochemicals from the methanol extract of *A. gangetica* using standard and chromatographic methods

Materials and Methods

Collection, identification and preparation

A. gangetica plant was collected in the September in Ugbowo, Benin City with latitude of 6^{o} 21' 1"N and longitude 5^{o} 36' 36"E. It was identified in the Department of Plant Biology and Biotechnology by H.A. Akinnibosun (Prof) with herbarium number UBH-460 assigned and the sample specimen was deposited in the herbarium of the Department of Plant Biology and Biotechnology. The leaves were detached, air-dried under-shade for two weeks and then pulverized by electric milling machine to powder. One hundred grams of the powder was macerated with 400 mL of methanol (99 %) for 72 hours. This was concentrated in vacuum at

50 °C and extract kept at 4 °C until used.

Phytochemical screening of the powdered leaves of *Asystasia gangetica*

Phytochemicals in the leaves of *A. gangetica* were determined by methods described by Sofowora and Trease and Evans. They include alkaloids, tannins, steroids, saponins, flavonoids, glycosides and terpenoids [20,21].

Test for Alkaloids:

0.5 g of the powdered leaves of *A. gangetica* was dissolved with 30 mL of HCl (diluted) and was filtered before testing for the presence of alkaloids. Mayer's test: Addition of four drops of Mayer's reagent to 1 mL of the filtrate produced a creamy-yellow precipitate indicates the presence of alkaloids. Wagner's test: Addition of four drops of Wagner's reagent to 1 mL of the filtrate in a test tube with the resultant formation of reddish-brown precipitate indicated the presence of alkaloids.

Test for Flavonoids and Saponins:

0.5 g powdered leaves of *A. gangetica was* dissolved in 30 mL of distilled water and boiled for 5 minutes. This mixture was then filtered and tested for flavonoids and saponins. Flavonoids: Addition of 4 drops of lead acetate to 1 mL of the filtrate in a test tube, with subsequent formation of a yellow colour precipitate, which indicate the presence of flavonoids. Saponins: Equal volume of filtrate and distilled water was vigorously shaken for 3 minutes. Saponins are indicated with the formation of persistent froth.

Test for Tannins:

0.5 g of powdered *A. gangetica* leaves was dissolved in 30 mL of distilled water. This mixture was heated for 20 minutes on a water bath and then filtered. Four drops of Ferric chloride was added to 1 mL of the filtrate in a test tube. Tannins are indicated by the production of dark green precipitate.

Test for Steroids:

To 30 mL of methanol in a test tube, 0.5 g of powdered leaves *A. gangetica* was added. This mixture was heated on a water bath for 30 minutes and then filtered while hot. Four drops of acetic anhydride were added to 1 mL of the filtrate. Production of violet to blue colour solution indicate the presence of steroids.

Test for Terpenoids:

Chlorofoerm (2 mL) was added to 10 mg of powdered leaves of A. gangetica in a test tube, and a layer of concentrated sulfuric acid (3 mL). Terpenoids are indicated by reddish-brown colouration of the sulfuric acid layer.

Test for glycosides:

Bontrager's Test: Ten percent of hydrochloric acid (20 mL) was utilized in boiling 5 mg of the powdered leaves of *A. gangetica* for 5 minutes on water bath, the mixture was filtered and cooled to room temperature. The resultant filtrate was diluted with equal volume of chloroform before adding 4 drops of 10 % ammonia and heat. Glycosides are indicated with the formation of pink colouration.

Gas Chromatography-Mass Spectrometric analysis of the methanol extract of *Asystasia gangetica*

GC-MS QP2010 SE model (Schmadzu, Japan) was utilized to analyze methanol extract of *A. gangetica*. Phases in the equipment include phenylmethylsiloxane (stationary pahse) and helium (mobile phase). Column (DB 5MS) of measurements (0.25 mm x 30 mm x 0.10 µm) and sample size of 1 µm was injected in the split mode. The operating conditions: inlet temperature 250 °C, oven temperature 60 °C for 3.4 min which was remped for 12 °C/min to 240 °C. Rate of increase was maintained until temperature changed to 290 °C and kept for 2 min. Electron impact mode with ionization energy of 70 eV was used for the mass

spectrometer and scanned within 45-700 dalton. Chemstation software was used to acquire data and compounds were identified by comparing the fragmentation patterns produced by each compound with data from the National Institute of Standard Technology [22].

High Pressure Liquid Chromatography Analysis of methanol extract of *A. gangetica*

Analysis (HPLC) of the methanol extract of *A. gangetica* was done using Shimadzu LC-10AD dual binary pumps, Shimadzu CTO-10AS column oven, and Shimadzu Prominence SPD-20A UV/Vis detector. C-12 normal phase column (Phenomenex, Gemini 5 µ, 200 mm length × 4.8 mm internal diameter) was utilized for the analysis. Mobile phase consisting of solvent A and B, Solvent A is made of acetic acid-acidified deionized water at pH 2.8, while solvent B is acetonitrile at 0.8 mL/min flow rate. Solvent B (5 %) was used to equilibrate the column for 20 min post injection of each sample. Temperature of the column was set at 38 °C, volume of injection was 20 µL and wavelength set at 280 nm, Compounds were identified and quantified by comparison of the retention times and peak areas with standard (pure) compounds by plotting calibration plot of external standards.

Gradient elution: 0-5 min, 5-9 % solvent B; 5-15 min, 9 % solvent B; 15-22 min, 9-11 % solvent B; 22-38 min, 11-18% solvent B; 38-43 min, 18-23 % solvent B; 43-44 min 23-90 % solvent B; 44-45 min, 90-80 %, solvent B; 45-55 min [23].

Results and Discussion

Preliminary phytochemical screening showed the presence of alkaloids, steroids, saponins, tannins, terpenoids, flavonoids and glycosides (**Table 1**) in the leaves of A. *gangetica*. Study carried out on leaves sample of *A. gangetica* collected from Obio/Akpor in River state showed the presence of tannins, cyanogenic glycosides and saponins [24]. This is in agreement with our study even though the samples were collected in different places. Location, altitude of the area, seasonal variation and exposure to pollution are important determinant in phytochemicals produced in a plant [25]. Similarly, flavonoids, alkaloids, glycosides, saponins and tannins have been reported in the flower of *A. gangetica* [26], implying that different parts of the plant could produce similar classes of compound, with similar pharmacological activity. Related phytochemicals have been reported in *A. variabilis* [27], indicating that the phytochemicals are not specific to *A. gangetica* but also seen in other species of the same genus.

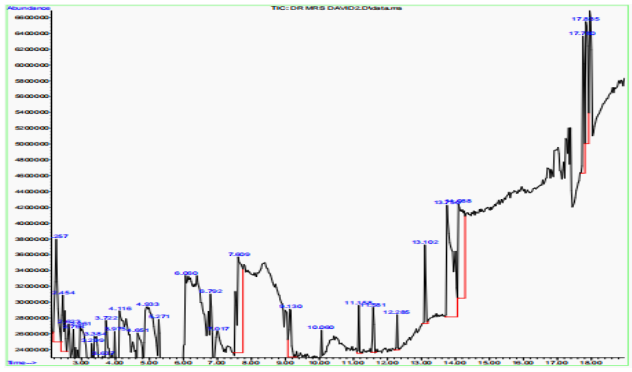

Figure 1*: Chromatogram of the GC-MS analysis of the methanol extract of A. gangetica*

Table 1 Phytoconstituents of the powdered leaf of *A. gangetica*

Phytochemical	Inference
Alkaloid	+
Saponin	+
Steroid	+
Tannin	+
Terpenoid	+
Flavonoids	+
glycoside	+

In this study, the leaves of *A. gangetica* were subjected to maceration with methanol after pre-treatment. This was utilized due to its

simplicity, low cost and environmental friendliness. When compared with Soxhlet method of extraction, the yield of the phytochemicals are high (maximum) and low energy consumption was utilized. Twenty-eight compounds were identified from the methanol leaves extract of *A. gangetica* (**Fig. 1**), most of which are esters, fatty acids, alcohols and imidazole derivatives (**Table 2**). Chromatographic profiling is an important tool for comparing the sample composition and complexity of the chemicals [28]. In this case, the GC-MS analysis, was used to determine the chemical nature of the volatile contents in the leaves of *A. gangetica*. Previous analysis of *A. travancorica* revealed ten compounds from the ethanol extract of the whole plant, out of which 2,6,10-Dodecartrien-1-ol and 9-hexylheptadecane were identified [29]. Also quercetin, ungeremine, lucenin, isoquinoline and cervnomycin were identified from the root extract of *A. gangetica* [19]. 2,3-Dihydroxypropyl elaidate initially identified in Tiger Milk mushroom which was shown to have significantly reduce IgE in serum and IL-13, IL-4 and IL-5 in bronchioalveolar lavage fluid was identified in the leaves of *A. gangetica*[30]. Ribitol (adonitol), is a crystalline pentose alcohol, largely seen in *Adonis vernalis*. It encourages the use of glucose in the glycolysis cycle, increase level of reduced gluthione and enhance nucleotide biosynthesis. These could be utilized in the developing of possible target for breast cancer therapy [31]. Some of the fatty molecules such as 7-octenoic acid; 13-docosenoic acid methyl ester; oleic acid; dodecanoic acid methyl ester; n-decanoic acid; octadecane, 6-methyl; octadecanoic acid; eicosanoic acid and 5-octadecene, (E), from the methanol extract of *A. gangetica* have been reported to have antioxidant activity and due to their ability to scavenge for free radicals, they could be used as preservative. Also the have been associated with antibacterial activity [32].

HPLC analysis revealed nineteen compounds (**Fig 2**) with Spartein (7.64 %), cyangenic glycoside (10.51 %), aphylidine (10.33 %) and flavone (7.51 %) as the prominent compounds (**Table 3**) he compounds identified can be grouped into flavonoids (kaempferol, naringenin, proanthocyanidine (oligomeric flavonoid), anthocyanin, flavone, flavonone), alkaloids (spartein, ribalinidine, ammodendrine, epehidrine, aphyllidine, dihydrocytisine), steroids, antinutrients (oxalate, phytate and tannins), glycoside (cyanogenic) and saponins. Kaempferol, isorhamnetin, quercetin and luteolin have previously beenreported in the whole plant by Gopal and co-workers [33].

Table 2 Phytoconstituents of methanol leaves extract of *A. gangetica*

S/N	Compounds	RT (min)	% Area	MF	MW
1	Ribitol	2.257	4.23	$C_5H_{12}O_5$	152.15
2	Tetradecanoic acid, propyl ester	2.454	2.17	$C_{17}H_{34}O_2$	270.45
3	cis-9-Hexadecenoic acid	2.623	1.05	$C_{16}H_{30}O_2$	254.41
4	Ribitol, 1,3:2,4-di-O-benzylidene-d-threitol	2.764	1.08	$C_{18}H_{18}O_4$	298.30
5	5-Octadecene, (E)-	2.961	3.48	$C_{18}H_{36}$	252.50
6	Eicosanoic acid	3.299	0.88	$C_{20}H_{40}O_2$	312.54
7	1-Thia-2-azacyclopenta[a]anthracene-3,6,11-trione	3.384	2.92	$C_{15}H_7NO_3S$	281.28

8	Tricosane, 2-methyl-	3.637	0.88	$C_{24}H_{50}$	338.65
9	Octadecanoic acid	3.722	2.70	$C_{18}H_{36}O_2$	284.48
10	Methyl 21-methyl-hexacosanoate	3.975	0.96	$C_{28}H_{56}O_2$	424.70
11	15-Hydroxypentadecanoic acid	4.651	2.63	$C_{15}H_{30}O_3$	258.40
12	Octadecane, 1-(ethenyloxy)-	4.933	8.77	$C_{20}H_{40}O$	296.53
13	Octadecane, 6-methyl-	5.271	1.65	$C_{19}H_{40}$	268.5
14	Tetraethyl 1,1'-(1,8-naphthylene)bis(1,2,3-triazole-4,5-dicarboxylate)	5.891	0.59	$C_{26}H_{26}N_6N_8$	550.5
15	15-Hydroxypentadecanoic acid	6.060	11.94	$C_{15}H_{30}O_3$	258.40
16	n-Decanoic acid	6.792	3.30	$C_{10}H_{20}O_2$	172.26
17	Dodecanoic acid, methyl ester	7.017	2.46	$C_{14}H_{28}O_2$	228.37
18	Z-(13,14-Epoxy)tetradec-11-en-1-olacetate	7.609	8.72	$C_{16}H_{28}O_3$	268.39
19	2,3-Dihydroxypropyl elaidate	9.130	1.85	$C_{21}H_{40}O_4$	356.5
20	17-Pentatriacontene	10.060	0.51	$C_{35}H_{70}$	490.90
21	Oleic Acid	11.158	0.69	$C_{18}H_{34}O_2$	282.5
22	D-erythro-Pentose, 2-deoxy-	11.581	1.12	$C_5H_{10}O_4$	134.13
23	Methyl 6-O-[1-methylpropyl]-beta.-d-galactopyranoside	12.285	0.53	$C_{11}H_{22}O_6$	250.00
24	13-Docosenoic acid, methyl ester	13.102	1.64	$C_{23}H_{44}O_2$	352.60
25	cis-Vaccenic acid	13.750	10.20	$C_{18}H_{34}O_2$	282.46
26	Cycloheptano[d]imidazolidine, 1,3-dihydroxy-2-methyl-	14.088	9.18	$C_9H_{18}N_2O_2$	186.25
27	7-Octenoic acid	17.750	3.71	$C_{10}H_{18}O_2$	170.25
28	Trehalose	17.835	4.81	$C_{12}H_{22}O_{11}$	342.30

Key: RT=Retention Time, % Area=Percentage Area, MF=Molecular Formula, MW=Molecular Weight

Kaempferol is a 3, 4', 5, 7-tetrahydroflavone, a natural flavonol, that have been identified in several plants. Possesses antioxidant, anti-inflammatory, neuroprotective, cardiovascular, chemopreventive and antimicrobial potentials [34, 35].T. Exert it chemopreventive action by blocking DNA damage at early stage (initiation step) or through arrest or reversal of the process at the progression and promotion steps [36]. Anthocyanins are water soluble vacuolar pigment, produced from the phenylpropanoid pathway. They produce characteristic colours (red or blue) in vegetables, like other polyphenols they possess the ability to scavenge for reactive oxygen and nitrogen species [37]. Naringenin is a 2, 3-dhydro-5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one. It is a flavanone that is derive from narirutin hydrolysis, apart from its ability to scavenge for free radicals, it modulate immune response potential [38]. Proanthocyanidine oligomeric flavonoid, derived from the condensation of two flavan-3-ol subunits by one single or double bond. In plants, they act as biochemical defense against external aggressors, making it effective

against fungi [39].These flavonoids are important to the plant either by impacting colour, flavour or fragrance. In human, they could alter vital biochemical pathways in the body, thus improving pathological conditions. However, they could act as preventive molecules or cause reversal of a debilitating condition. Moreso, oxalates, tannins and phytates that are classified as anti-nutrient, it can affect the absorption of nutrients when not properly prepared or when consumed in high quantity. However it has some beneficial effects, as seen in its anti-inflammatory and antioxidant potentials [40].

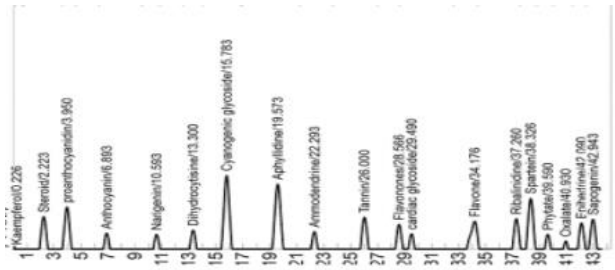

Figure 2: *Chromatogram for the HPLC analysis of the methanol extract of A. gangetica.*

Table 3 Phytoconstituents from the HPLC analysis of the methanol leaf extract of *A. gangetica*

S/N	Compound	Retention Time (min)	Area (%)	Concentration (µg/ml)
1	Kaempferol	0.226	1.29	1.8214
2	Steroids	2.223	5.56	4.6952
3	Proanthocyanidine	3.950	6.69	13.9885
4	Anthocyanin	6.893	3.68	5.7657
5	Naringenin	10.593	3.55	5.5688
6	Dihydrocytisine	13.300	4.03	6.3206
7	Cyanogenic glycoside	15.783	10.51	8.9749
8	Aphylidine	19.573	10.33	0.8215
9	Ammodendrine	22.293	3.90	2.3273
10	Tannin	26.000	5.58	3.6986
11	Flavonone	28.566	4.75	9.9310
12	Cardiac glycoside	29.490	3.65	5.5250
13	Flavone	34.176	7.51	11.3604
14	Ribalinide	37.260	5.38	8.1357
15	Spartein	38.326	7.64	11.5595
16	Phytate	39.590	3.42	5.1781
17	Oxalate	40.930	2.59	1.1912
18	Ephedrine	42.090	4.70	7.0800
19	Sapogenin	42.943	5.26	7.9606

Sparteine is a quinolizidine alkaloid cause slight analgesia, reduce motility and act as anticonvulsant against acute seizure and status epilepticus [41]. Other effects associated with sparteine include reduction in cardiac conductivity, respiratory arrest, circulatory collapse and stimulation of uterine motility [42]. Ribalinidine is a tertiary alkaloid with 4-quinolone framework have shown radical scavenging potential [43]. Ammodendrine is a piperidine alkaloid known as 1-[5-[(2R)-piperidin-2-yl]-3,4-dihydro-2H-pyridin-1-yl]ethanone with acute murine toxicity[44]. Ephedrine is a central nervous system stimulant, use to treat narcolepsy, asthma and obesity [45]. Worthy of note, is that the ethno-medicinal usage of the plant could be due to the synergistic or additive effects of the individual compounds.

Conclusions

A. gangetica powdered leaves contain phytochemicals such flavonoids with antioxidant, cardioprotective, antimicrobial,

chemopreventive and anti-inflammatory potentials. Also it contains alkaloids with analgesic and anticonvulsant potentials, radical scavenging activity and as CNS stimulant. Other phytochemicals identified include phytates, saponins, tannins and oxalates that could affect the bioavailability of nutrients, thus could be used as anti-obesity agent. This thus validate some of the ethnomedicinal usage of *A. gangetica*.

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Author's contribution statement

E. E. Odion: Conceptualization, Writing of the Original manuscript-editing, Supervision; **D. E. Elakhe:** Data collection, Writing-review and editing; **C. C. Osigwe:** Data collection, Writing-review and editing, Supervision; **D. A. Ambe:** Data collection, Writing-review and editing, Supervision; **E. C. Odiete:** Data collection, Writing-review and editing, Supervision

Conflict of Interest

All author declared no conflict of interest

Data Availability Statement

The data that support the findings of this study can be made available from the corresponding author, upon reasonable request.

References

1. S. Ismail and A, Shukor, Effects of water stress, shading and clipping on growth and development of *Asystasia gangetica, Plant Protection Quarterly*, 1998, 13, 140-142.

2. P. Acevedo-Rodríguez, Vines and Climbing Plants of Puerto Rico and the Virgin Islands. Contributions from the United States National Herbarium, 2005, 51:483.

3. F.H.K. Asiedu, E.N.W. Oppong and A.A. Opoku, Utilisation by sheep of herbage under tree crops in ghana. trop. *Animal Health Production*, 1978, 10, 1–10.

4. P.M. Maundu, G.W. Ngugi and C.H.S. Kabuye, Traditional Food Plants of Kenya. Nairobi: Kenya Resource Centre for Indigenous Knowledge (KENRIK), National Museums of Kenya, 1999.

5. T. Setyawati, S. Narulita, I.P. Bahri and G.T. Raharjo, A Guide Book to Invasive Alien Plant Species in Indonesia. Edited by Tukirin Partomihardjo, Soekisman Tjitrosoedirdjo, and Sunaryo. Research, Development and Innovation Agency Ministry of Environment and Forestry Republic of Indonesia. Bogor, 2015.

6. T-W. Hsu, T-Y. Chiang and J-J Pe. *Asystasia gangetica (L.) T.* Anderson subsp. micrantha (Nees) Ensermu (Acanthaceae), A Newly Naturalized Plant in Taiwan. *Taiwania*, 2005, 50(2), 117-122.

7. Jstor Plant Science, *Asystasia gangetica* synonyms: http://plants.jstor.org/taxon/synonymy/Asystasia.gangetica, Retrieved 28 July 2010.

8. K. Yeshi, D. Crayn, E. Ritmejerytė and P, Wangchuk. Plant secondary metabolites produced in response to abiotic stresses has potential application in pharmaceutical product development, *Molecules,* 2022, 5; 27(1):313. (DOI: 10.3390/molecules27010313)

9. S.K. Tilloo, V.B. Panda, T.M. Rasala and V.V. Kale. *Asystasia gangetica*: Review on Multipotential, Application. *International Journal of Pharmacy*, 2012, 3(4), 18-20.

10. J.E. Kokwaro. Medicinal plants of East Africa. General Printers Limited Kenya, 1976. 12.

11. P.A. Akah, K.S. Gamaniel, A.Samson and C.O. Wambebe, Evaluation of nigeria traditional medicine. effect of gakani, a herbal antiasthmatic drug. *Journal of Ethnopharmacology*, 1997, 53, 87-93.

12. O.O. Adeyemi, F.R. Aigbe and N.G. Uyaiabasi, Analgesic and anti-inflammatory activities of the aqueous stem and leaf extract of *Asystasia gangetica* (Linn) T. Anderson, Nigerian Quaterly *Journal of Hospital Medicine*, 2011, 21(2), 129-34.

13. P. Mugabo and I.A. Raji, Effects of aqueous leaf extract of *Asystasia gangetica* on the blood pressure and

heart rate in male spontaneously hypertensive wistar rats, BMC *Complementary and Alternative Medicine*, 2013, 13, 283, (DOI: doi.org/10.1186/1472-6882-13-283)

14. I.O. Eriamiatoe, M.O. Edema, T. Eriamiatoe and G.E. Okpara, Chemical Constituents and Pharmacological use of *Asystasia gangetica* (Chinese Violet) as an Antiulcer Plant, *Journal of Chemical Society of Nigeria*, 2020, 45(2),324-336.

15. E.D. Daffodil, M.L. Packia, E.D. Pon and V.R. Mohan, Pharmacochemical characterization and antibacterial activity of *Asystasia gangetica* (L.) T. And, *Journal of Harmonized Research in Pharmacy*, 2013, 2(2), 112-120.

16. M.Y.U. Barbaza, K.A.D. Cruz, C-L. Hsieh, P-W. Tsai. Determination of the Chemical Constituent Contents and Antioxidation Properties of *Asystasia gangetica. Indian Journal of Pharmaceutical Education and Research*, 2021, 55(3) 863-871.

17. P.A. Akah, A.C. Ezike, S.V. Nwafor, C.O. Okoli, N.M. Enwerem. Evaluation of the anti-asthmatic property of *Asystasia gangetica* leaf extracts. *Journal of Ethnopharmacology* 89(1), 2003 25-36. (DOI: 10.1016/s0378-8741(03)00227-7)

18. D.M. Olufunke, Essential Oils from Aerial, Seed and Root of Nigerian *Asystasia gangetica* (L), *Journal of Essential Oil Bearing Plants*, 2011, 14(5), 582-589. (DOI: 10.1080/0972060X.2011.10643975)

19. V. Tamilselvan, M. Rajeswari and P. Velayutham, GC-MS Analysis and Invitro Anticancer Activity of Methanolic Root Extract of *Asystasia gangetica* (L.), *World Journal of Pharmacy and Pharmaceutical Sciences*, 2014,3(12), 957-967

20. W.C. Evans, Trease and Evans Pharmacognosy, 16th Edition, Elsiver Limited, 2009, 135-147.

21. A. Sofowora, Phytochemical Screening of Medicinal Plants and Traditional Medicine in Africa Edition, Spectrum Books Ltd., Nigeria, 1993, 150-156.

22. E.E. Odion, R.O. Ogboru and M.O. Ighene, Identification of Compounds in *Elaeis guineensis* Fruits using GC-MS, *Dhaka University Journal of Pharmaceutical* Science, 2020, 19(2), 153-159. (DOI: doi.org/10.3329/dujps.v19i2.50631)

23. O. Kaisoon, S. Siriamornpun, N. Weerapreeyaku and N. Meeso, Phenolic Compounds and antioxidant activities of edible flowers from Thailand,*Journal of Functional Foods*, 2011, 3:88–99.

24. E.C. Chuku, O.S. Chuku and M.G. Ajuru, Studies on the propagation, phytochemical properties, storage, utilization and shelf-life of *Asystasia gangetica*, *Current Studies In Comparative Education, Science And Technology* 5 & 6, 2019, 1-2, 135-142

25. S.S. Gololo. Effects of Environmental Factors on the Accumulation of Phytochemicals in Plants. In book: Phytochemistry. 3 marine sources, industrial applications, and recent advances. publisher: Apple Academic Press Inc, 2019.

26. K. Sama, R. Sivaraj, H. A. Salam and P. Rajiv, Pharmacognostical and phytochemical screening of *Asystasia gangetica* (Chinese violet), *International Research Journal of Pharmacy*, 2013, 4(2), 161-163.

27. R. Wijerathna, N.A.V. Asanthi, W.D. Ratnasooriya, R.N. Pathirana and N.R.M. Nelumdeniya, Evaluation of *In-vitro* antibacterial activity and phytochemical profile of aqueous leaf extract of *Asystasia variabilis, Journal of Pharmacognosy and Phytochemistry*, 2018, 7 (3),639-642.

28. J. C. Rogers, L. S. Winkler and M. F. Borgerding, Chromatographic profiling as a tool in the comparison and evaluation of complex mixtures, *Journal of Chromatographic Science*, 1997, 35(5), 193-200 (DOI: doi.org/10.1093/chromsci/35.5.193)

29. T. Komalavalli, P, Lincy, S. Muthukumarasamy and V. R. Mohan, Determination of bioactive components of *Asystasia travancorica* Bedd (Acanthaceae) by GC-MS analysis, *International Journal of Pharmaceutical and Clinical Research*, 2014, 6(2), 155-158

30. M. Johnathan, S.H.Gan, M.F. Ezumi, A.H. Faezahtu and A.A. Nuru, Phytochemical profiles and inhibitory effects of tiger milk mushroom *(lignosus rhinocerus*) extract on ovalbumin-induced airway inflammation in a rodent model of asthma, *BMC Complementary and*

Alternative Medicine, 2016,3;16:167. (DOI: 10.1186/s12906-016-1141-x)

31. J.D. Tucker, R. Doddapaneni, P.J. Lu and Q.L. Lu. Ribitol alters multiple metabolic pathways of central carbon metabolism with enhanced glycolysis: A metabolomics and transcriptomics profiling of breast cancer. *PLoS One.* 2022, 7; 17(12):e0278711. (DOI: 10.1371/journal.pone.0278711)

32. L. Gomez, B. Tiwari and M. Garcia-Vaquero, Chapter 9 - Emerging extraction techniques: microwave-assisted extraction. sustainable seaweed technologies cultivation, biorefinery, and applications *Advances in Green and Sustainable Chemistry* 2020, 207-224 (DOI: https://doi.org/10.1016/B978-0-12-817943-7.00008-1)

33. T.K. Gopal, G. Megha., D. Chamundeeswari and C.U. Reddy, Phytochemical and pharmacological studies on whole plant of *Asystasia gangetica, Indian Journal of Research in Pharmacy and Biotechnology*, 2013, 1(3), 365-370.

34. S. Archoo, S.H. Naikoo and S A. Tasduq, Role of herbal products as therapeutic agents against ultraviolet radiation-induced skin disorders, *Herbal Medicines. A Boon for Healthy Human Life*, 2022, 345-360.

35. H.S. Lee, H.J. Cho, R. Yu, K.W. Lee, H.S. Chun and J.H.Y. Park, Mechanisms underlying apoptosis-inducing effects of kaempferol in HT-29 human colon cancer cells, *International Journal of Molecular Science*, 2014, 15(2), 2722–2737.

36. G.J. Kelloff, C.C. Sigman and P. Greenwald, Cancer chemoprevention, progress and promise, *European Journal of Cancer*, 1999, 35, 2031–2038

37. R. Mattioli, A. Francioso, L. Mosca and P. Silva, Anthocyanins: A Comprehensive review of their chemical properties and health effects on cardiovascular and neurodegenerative diseases, *Molecules*, 2020, 21;25(17), 3809. (DOI: 10.3390/molecules25173809.

38. B. Salehi, P.V.T. Fokou, M. Sharifi-Rad, P. Zucca, R. Pezzani, N. Martins and J. Sharifi-Rad, The therapeutic potential of naringenin: a review of clinical trials, *Pharmaceuticals (Basel)*, 2019, 10;12(1),11. (DOI: 10.3390/ph12010011.

39. G. Mannino, G. Chinigò, G, Serio, T. Genova, C. Gentile, L. Munaron and C.M. Bertea, Proanthocyanidins and where to find them: a meta-analytic approach to investigate their chemistry, biosynthesis, distribution, and effect on human health, *Antioxidants (Basel)*, 2021, 30 10(8),1229, (DOI: 10.3390/antiox10081229)

40. M. López-Moreno, M. Garcés-Rimón and M. Miguel, Antinutrients: lectins, goitrogens, phytates and oxalates, friends or foe, *Journal of Functional Foods*. 2022, 89, 104938.
(DOI: https://doi.org/10.1016/j.jff.2022.104938)

41. F. Villalpando-Vargas and L. Medina-Ceja, Sparteine as an anticonvulsant drug: evidence and possible mechanism of action, *Seizure,* 2016, 39, 49-55. (DOI: 10.1016/j.seizure.2016.05.010)

42. J.K. Aronson, Meyler's Side Effects of Drugs, *The International Encyclopedia of Adverse Drug Reactions and Interactions, Reference Work*, 16th Edition, 2016.

43. M.B. Rahmani and M.A.B. Sukari, New lignum and other chemical components from *Haplophyllum villosum* and *H. leaviusculum* and their antioxidant Activity, *Proceedings of the 16th Malaysian Chemical Congress,* Malaysia, 2010.

44. S. T. Lee, R. J. Molyneux, C-W. T. Chang, D.R. Gardner, J.A. Pfister, K.E. Panter, and M. Garrossian, Ammodendrine and N -Methylammodendrine enantiomers: isolation, optical rotation, and toxicity, *Journal of Natural Products,* 2005, 68(5):681-5. (DOI: 10.1021/np0580199)

45. ASHSP, Ephedrine", The American Society of Health-System Pharmacists. Archived from the original on 2017-09-09, Retrieved 8 September 2017. Ephedrine". The American Society of Health-System Pharmacists. Archived from the original on 2017-09-09, Retrieved 8 September 2017