PRINT ISSN 1119-8362
Electronic ISSN 2659-1502

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 27 (3) 543-548 March 2023

Evaluation of Phytochemical Constituents of Fresh Roots Aqueous and Ethanolic Extracts of the Medicinal Plants of *Uvaria chamae*, *Anthocleista djalonensis* and *Euadenia eminens*

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ABSTRACT: Phytochemicals are bioactive plant chemicals nutrients typically found in fruits, vegetables, grains, and other plant foods which may provide desirable health benefits beyond basic nutrition and can help reduce risks of major chronic diseases. This study was conducted to evaluate the phytochemical constituents of fresh roots ethanolic and aqueous extracts of medicinal plants of *Uvaria chamae*, *Anthocleista djalonensis* and *Euadenia eminens* using standard methods of phytochemical screening and Gas Chromatography - Mass Spectrometry (GC-MS) analysis. Phytochemical screening revealed a diverse array of phytochemical constituents such as alkaloids, flavonoids, phenols, tannins, saponins, isoprenoids, steroids, phlobatanins and cardiac glycosides. The results of GC-MS analysis of aqueous and ethanolic extracts of *U. chamae* revealed the presence of four and fourteen compounds respectively. Aqueous and ethanolic extracts of *A. djalonensis* had ten and seventeen compounds respectively while the aqueous and ethanolic extracts of *E. eminens* was found to have six and twenty compounds respectively. These results indicate *U. chamae, A. djalonensis* and *E. eminens* possess phytochemicals which have potential antioxidant, antidiabetic, anticancer, anti-inflammatory activity amongst others and as such, can be recommended as plants of phytopharmaceutical importance.

DOI: **https://dx.doi.org/10.4314/jasem.v27i3.18**

Cite this paper as: OKEREKE, A; SANNI, O; MBONU, J; ONUOHA, O; TIAMIYU, O. A; ADEWOLE, A. R; OKPUZOR, J (2023). Evaluation of Phytochemical Constituents of Fresh Roots Aqueous and Ethanolic Extracts of the Medicinal Plants of *Uvaria chamae*, *Anthocleista djalonensis* and *Euadenia eminens*. *J. Appl. Sci. Environ. Manage.* 27 (3) 54-548

Dates: Received: 12 February 2023; Revised: 12 March 2023; Accepted: 23 March 2023
Published: 31 March 2023

Keywords: *Uvaria chamae*; *Anthocleista djalonensis*; *Euadenia eminens*; Ethanolic Extracts; Medicinal Plants

Plants provide not only essential nutrients needed for life, but also other bioactive phytochemicals that contribute to health promotion and disease prevention (Yoo *et al.*, 2018). Medicinal plants refer to a variety of plants that have medicinal properties. These plants are a rich source of compounds that can be used to develop drugs. The parts of medicinal plants that may be used are seeds, root, leaf, fruit, skin, flowers or even the whole plant (Jamshidi-Kia *et al.*, 2018). Phytochemicals, often called secondary metabolites, are non-nutritive chemical compounds produced by plants via several chemical pathways (Yoo *et al.*, 2018). *Uvaria chamae* is a climbing medicinal plant that belongs to the family Annonaceae and is commonly found in West Africa, where it is known with different names by the Igbo, Hausa, Yoruba, Esan, and Igala natives of Nigeria as *Mmimi ohia*, *Kaskaifi*, *Oko oja*, *Ogholo*, and *Ayiloko* respectively (Emeka *et al*., 2015). *U. chamae* is used traditionally to treat diabetes mellitus and other conditions such as

bronchitis, gastroenteritis, amenorrhea, menorrhagia, abdominal pain and wound healing. Several studies have confirmed that the bioactive compounds of *U. chamae* such as alkaloids, flavonoids, phenols, tannins, and terpenoids produce hypoglycemic, anti-inflammatory, antifungal, and antimalarial effects (Emordi *et al*., 2018). *Anthocleista djalonensis* A. Chev is a shrubby-like tree belonging to the family Gentianaceae and broadly distributed in Nigeria and the West African sub-region. Several parts of the plant are commonly used for different medicinal reasons in Nigeria and other western countries (Ezirim *et al*., 2019). *A. djalonensis* has been reported to have anti-diabetes, antimalarial, anti-pyretic, anthelmintic, antimycobacterial, anti-bacterial and wound healing properties (Awah *et al*., 2010). Traditionally, it has been used as a laxative, purgative and contraceptive and also in the treatment of a wide range of illnesses including stomach aches, diabetes, malaria, fever, constipation etc (Anyanwu *et al*., 2015). *Euadenia eminens* of the Capparaceae family was given this name by Joseph Dalton Hooker in 1881. It is found chiefly in Ghana and Sierra Leone, and in other West African countries. *E. eminens* is locally known as *Akwukwo ato* in the eastern part of Nigeria, and *Wowo* in the western part of Nigeria. The juice from its cut fruit is used as drops for sore eyes, conjunctivitis, iritis, ophthalmia and trachoma. The fruit pulp is eaten as an aphrodisiac (Dickson *et al*., 2012). This study therefore seeks to evaluate the phytochemical constituents of fresh roots ethanolic and aqueous extracts of medicinal plants of *Uvaria chamae, Anthocleista djalonensis* and *Euadenia eminens.*

MATERIALS AND METHODS

Plant Material and Collection: Fresh roots of *Uvaria chamae, Anthocleista djalonensis* and *Euadenia eminens* were purchased from Mushin market, Lagos state. *U. chamae* and *A. djalonensis* were identified and authenticated at the Herbarium of the Department of Botany, University of Lagos, Nigeria, with voucher numbers LUH: 7021 and LUH: 7022 respectively while *E. eminens* was identified at the botanical unit of the School of Biological Sciences, Abia State University, Uturu, Nigeria by Dr. O. Ojimgba.

Extraction of Crude Extract: The fresh root barks of *U. chamae, A. djalonensis* and *E. eminens* were washed, chopped into small pieces, weighed and air-dried at room temperature. The dried roots of each plant were then ground into coarse powder using a Christy & Norris Laboratory Mill. Extraction was done by dispersing and soaking each powdered plant material in distilled water and 80% ethanol, at sample to solvent ratio of 1:10, separately for 72 hours with intermittent agitation to ensure proper mixing of plant sample with solvents for accurate extraction. The resulting extracts were filtered using muslin cloth, cotton wool and Whatman No. 1 filter paper and filtrates were concentrated to dryness with the aid of a water bath at 40^0C. The final weight of the extract was noted and the percentage yield was calculated with reference to the initial weight of the powder as given below:

$$\%\ yield\ = \frac{\text{FEW}}{\text{IWPS}} \times 100$$

Where FEW = final weight of extract and IWPS = initial weight of powdered sample.

The extracts were stored in air-tight containers in a refrigerator at 4 ^{0}C until required.

Phytochemical Screening: Qualitative and quantitative phytochemical analyses were performed using standard procedures as described by Trease and Evans (1989) to reveal the presence of various chemical constituents.

Gas Chromatography – Mass Spectrometry: GC-MS analysis of the plant extracts was carried out using Agilent Technologies GC-MS (GC-7890A, MS 5975C). Spectroscopic detection involved an electron ionization system which utilized high energy electrons (70eV). The injector temperature was set at 250 °C and pure helium gas (99.999%) was used as the carrier gas with flow rate of 2mL/min. The oven temperature was set at 80 °C for 2 min then increased to 240 °C at 10 °C/min. One microlitre of the prepared extracts was injected in splitless mode. Total run time was 32 minutes. The chromatograms of the sample were identified by comparing their mass spectra with NIST14.L library data, and the GC retention time against known standards.

RESULTS AND DISCUSSION

Percentage Quantitative Yield of Extracts: The yield of plant extracts from the selected medicinal plants under the influence of different solvent systems is shown in Table 1. Higher yield was obtained when ethanol was used as the solvent with *Euadenia eminens* as the exception.

Table 1: Percentage yield of the plant extracts

S/N	Crude Extract	% Yield of Aqueous Extract	% Yield of Ethanolic Extract
1	*Uvaria chamae*	1.2	6.4
2	*Anthocleista djalonensis*	2.4	3.0
13	*Euadenia eminens*	4.7	2.8

Phytochemical Analysis: Phytochemical screening of the plant extracts revealed a variety of phytochemical constituents such as alkaloids, flavonoids, phenols. tannins, saponins, steroids, phlobatanin and cardiac glycosides as shown in Table 2. Gas Chromatography – Mass Spectrometry (GC-MS): The GC-MS results of the aqueous and ethanolic extracts of the selected medicinal plants, as shown in table 3, revealed the presence of a diverse array of compounds. The compounds are listed in ascending order of retention time and their biological activity is also contained in the table 3.

Table 2: Phytochemicals detected in the plant extracts

		Alkaloids	Flavonoids	Phenols	Tannins	Saponins	Steroids	Phlobatanin	Cardiac Glycosides
Aqueous extract of *U. chamae*	Quality	+	+	+	+	+	+	+	+
	Quantity	19.25	41.86	28.72	11.58	22.26	21.52	29.1	8.4
Ethanolic extract of *U. chamae*	Quality	+	+	+	+	-	+	+	+
	Quantity	15.3	34.5	15	20.8	-	32.5	57	10.5
Aqueous extract of *A. djalonensis*	Quality	+	+	+	+	+	+	-	-
	Quantity	16.15±0.13	36.63±0.18	17.63±0.09	8.15±0.14	21.43±0.05	20.95±0.17	-	-
Ethanolic extract of *A. djalonensis*	Quality	-	+	+	+	+	-	-	-
	Quantity	-	40.90±0.23	28.20±0.16	15.62±0.12	17.65±0.13	-	-	-
Aqueous extract of *E. eminens*	Quality	+	+	+	-	+	-	+	-
	Quantity	30.36±0.27	33.11±0.14	12.99±0.26	-	19.99±0.03	-	69.06±0.21	-
Ethanolic extract of *E. eminens*	Quality	+	+	+	+	-	-	+	+
	Quantity	27.88±0.27	29.75±0.33	18.59±0.24	2.16±0.23	-	-	35.56±0.21	15.36±0.02

Key: + present, - absent

Table 3: Bioactive compounds detected in the extracts of the selected medicinal plants

EXTRACT	RT	Name of Compound	MF	MW	Peak Area (%)
Aqueous extract of *U. chamae*	4.365	n-Hexane	C_6H_{14}	86	49.590
	21.276	Pentanoic acid, 4-methyl, methyl ester	$C_7H_{14}O_2$	130	0.602
	22.698	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	34.056
	23.111	2-Methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	280	0.057
Ethanolic extract of *U. chamae*	8.111	D-Limonene	$C_{10}H_{16}$	136	60.141
	9.552	Cyclohexene, 1-methyl-5-(1-methylethenyl)	$C_{10}H_{16}$	136	1.669
	9.959	p-Mentha-1,8-dien-7-ol	$C_{10}H_{16}O$	152	3.477
	11.043	1(3H)-Isobenzofuranone, 3a,4,5,7a-tetrahydro-4-hydroxy-3a,7a-dimethyl-, (3a.alpha.,4.beta.,7a.alpha.)-(.+/-.)-	$C_{10}H_{14}O_3$	182	2.374
	11.794	Cis-p-mentha-1(7),8-dien-2-ol	$C_{10}H_{16}O$	152	3.352
	13.220	Copaene	$C_{15}H_{24}$	204	0.794
	13.989	Germacrene D	$C_{15}H_{24}$	204	1.698
	19.625	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	3.100
	21.288	Methyl 10-trans,12-cis-octadecadienoate	$C_{19}H_{34}O_2$	294	5.279
	21.450	Methyl 9-cis,11-trans-octadecadienoate	$C_{19}H_{34}O_2$	294	8.438
	23.826	2H-Pyran-2-one, 6-ethyltetrahydro	$C_7H_{12}O_2$	128	3.750
	24.242	Vinyl ethyl sulfoxide	C_4H_8OS	104	2.202
	25.094	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	$C_{16}H_{48}O_7Si_8$	577	1.222
	27.042	Ethanone, 2-(2-benzothiazolylthio)-1-(3,5-dimethylpyrazolyl)-	$C_{14}H_{13}N_3OS_2$	303	2.503
Aqueous extract of *A. djalonensis*	13.571	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-	$C_{15}H_{24}$	204	5.887
	18.594	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	8.166
	19.804	Phytol	$C_{20}H_{40}O$	296	14.757
	20.253	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	310	5.038
	23.583	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	4.584
	24.539	Tricosane	$C_{23}H_{48}$	324	5.486
	25.529	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	667	2.923
	25.694	Squalene	$C_{30}H_{50}$	410	18.990
	26.161	Nonacosane	$C_{29}H_{60}$	408	4.559

	29.239	(Phenylthio)acetic acid, 1-adamantylmethyl ester	$C_{19}H_{24}O_2S$	316	9.648
Ethanolic extract of *A. djalonensis*	4.106	Furan, tetrahydro-3-methyl-	$C_5H_{10}O$	86	12.941
	13.078	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	214	0.799
	13.671	Z-3-Hexadecen-7-yne	$C_{16}H_{28}$	220	0.299
	14.060	Caryophyllene oxide	$C_{15}H_{24}O$	220	1.042
	14.202	Diethyl Phthalate	$C_{12}H_{14}O_4$	222	0.803
	15.390	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	0.670
	17.494	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.506
	18.064	Benzene, 1-isothiocyanato-2-methyl	C_8H_7NS	149	1.712
	19.217	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	4.800
	20.150	6-Octadecenoic acid, (Z)-	$C_{18}H_{34}O_2$	282	16.803
	20.494	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	3.061
	20.921	2-(2-Thienyl) pyridine	C_9H_7NS	161	28.649
	22.565	Heptadecane	$C_{17}H_{36}$	240	3.267
	23.139	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	2.074
	24.092	Tetracosane	$C_{24}H_{50}$	338	1.200
	25.136	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	$C_{15}H_{26}O$	222	0.183
	25.595	Carbonic acid, decyl undecyl ester	$C_{22}H_{44}O_3$	356	0.783
Aqueous extract of *E. eminens*	7.906	D-Limonene	$C_{10}H_{16}$	136	66.697
	19.302	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	5.014
	21.000	10,13-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	12.286
	23.868	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	$C_{30}H_{48}O$	424	5.114
	26.374	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	$C_{17}H_{14}O_4$	282	6.632
	27.336	1H-Pyrazole, 1-(3-methylbutyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)	$C_{14}H_{25}BN_2O_2$	264	4.257
Ethanolic extract of *E. eminens*	17.477	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	3.427
	17.710	3-Eicosene, (E)-	$C_{20}H_{40}$	280	2.291
	19.898	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	9.325
	20.094	3,4-Dihydroxymandelic acid, 4TMS derivative	$C_{20}H_{40}O_5Si_4$	472	0.766
	20.178	Methyl stearate	$C_{19}H_{38}O_2$	298	1.411
	20.607	1-Docosene	$C_{22}H_{44}$	308	2.374
	21.279	Propan-2-ol, O-tert-butyldimethylsilyl ether	$C_9H_{22}OSi$	174	8.414
	21.499	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	$C_{13}H_{39}O_5Si_6$	444	2.663
	22.772	1-Tetracosene	$C_{24}H_{48}$	336	1.296
	24.210	2,5-Dihydroxybenzoic acid, 3TMS derivative	$C_{16}H_{30}O_4Si_3$	370	3.533
	24.461	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	14.027
	25.504	Tricosane	$C_{23}H_{48}$	324	6.871
	25.859	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	382	2.198
	26.404	Eicosane	$C_{20}H_{42}$	282	2.308
	26.632	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	667	2.454
	26.833	Squalene	$C_{30}H_{50}$	410	2.418
	27.455	Nonacosane	$C_{29}H_{60}$	408	12.269
	27.857	Hexacosanoic acid, methyl ester	$C_{27}H_{54}O_2$	410	1.894
	29.702	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$	741	2.023
	29.839	Docosane	$C_{22}H_{46}$	310	5.296

Medicinal plants have been used traditionally to treat a wide array of diseases. They are a rich source of secondary metabolites which are responsible for their therapeutic properties and as such can be seen as valuable resources for drug development. This study identified phytochemicals present in *Uvaria chamae*, *Anthocleista djalonensis* and *Euadenia eminens*. Phytochemical analysis of aqueous *A. djalonensis* revealed the presence of alkaloids, flavonoids, phenols, tannins, saponins and steroids. Similar results were shown by Leke *et al.* (2012). The result in this present study is in agreement with previous investigation of Thomas and Essien (2020), who reported the presence of phenols, tannins and cardiac glycosides in the ethanolic extract of *U. chamae*. Flavonoids were present in all the plant extracts and was the most abundant phytochemical in both the aqueous and ethanolic extracts of *A. djalonensis* as well as the aqueous extract of *U. chamae*. Flavonoids possess many biochemical properties such as anti-inflammatory, hepatoprotective, antibacterial, anti-cancer, antiviral activities but the best described

property of flavonoids is their capacity to act as antioxidants (Kumar and Pandey, 2013). Alkaloids have dynamic biological activities in human or animal body and have been reported to have anti-inflammatory, anti-plasmodic, antipsychotic, antihypertensive and hepatoprotective activity (Debnath *et al*., 2018). Phenols are widespread in nature and possess anti-microbial, anti-bacterial, antioxidant, pharmacological and nutritional properties. The antioxidant activity of natural phenols has been related to their scavenger ability towards free radicals (Floris *et al*., 2021). Tannins are a diverse group within phenolic compounds widely distributed in nature. The most relevant biological activities of tannins are antioxidant, anti-inflammatory, anti-diabetic, cardioprotective, healing and antimicrobial (antiviral and antibacterial) (Fraga-Corral *et al*., 2021). GC-MS analysis revealed the presence of a wide range of compounds, some of which have been previously reported to have therapeutic activities. D-Limonene was found in the ethanolic extract of *U. chamae* as well as the aqueous extract of *E. eminens* and was the most abundant compound in these extracts. It is a monocyclic monoterpene and the principal active form of limonene. It has been found to possess antioxidant, antidiabetic, anticancer, anti-inflammatory, cardioprotective, gastroprotective, hepatoprotective, immune modulatory, anti-fibrotic, anti-genotoxic effects amongst others (Anandakumar *et al*., 2021). Squalene is considered a potent chemopreventive and chemotherapeutic agent with antitumor, antioxidant and antibacterial activity (Sermakkani *et al*., 2012). Lozano-Grande *et al*. (2018) reported that it inhibits tumor growth in the colon, skin, lung, and breast and stimulates the immune system for the application of drugs in the treatment of diseases such as HIV, H1N1, leukemia, papilloma, and herpes. Copaene, a colorless and clear viscous liquid sesquiterpenes hydrocarbon, possesses antioxidant, anti-proliferative, antigenotoxic properties (Gogoi *et al*., 2020). Phytol and its derivatives exhibit a wide range of bioactivities including anti-anxiety, cytotoxic, metabolism-modulating, antioxidant, autophagy- and apoptosis-inducing, anti-nociceptive, anti-inflammatory, immune-modulating, and antimicrobial effects (Islam *et al*., 2018). It also gives good preventive and therapeutic results against arthritis (Tyagi and Agarwal, 2017). Hexadecanoic acid methyl ester was found in the ethanolic extracts of all the selected plants as well as the aqueous extract of *E.* eminens. It is a type of fatty acid ester which can inhibit the growth of pathogenic bacteria (Shaaban *et al*., 2021). It possesses antibacterial effects against bacteria such as *P. aeruginosa*, *B. subtilis* and *K. pneumoniae* (Lalthanpuii and Lalchhandama., 2019) and a good inhibitory effect against Gram-positive and Gram-negative bacteria (Davoodbasha *et al.,* 2018). Hexadecanoic acid, ethyl ester is a palmitic acid ethyl ester with antioxidant, hemolytic, hypocholesterolemic, antiandrogenic properties (Tyagi and Agarwal, 2017). Akpuaka *et al*. (2013) reported that Eicosane possesses antifungal, antibacterial, antitumor and cytotoxic effects while Docosane and Nonacosane possess antibacterial activity.

*Conclusion***:** In order to promote future development of new drugs in chemotherapeutics, extraction of bioactive components from the parts of the plant, determination of relative abundance, and detailed information on their bioactivity is very important. The literature data and the findings in this study confirms that the evaluated plants, well known in traditional medicine, possess various preventive and therapeutic benefits and provide potential sources of bioactive compounds. Moreover, given the number of phytochemicals isolated so far, nature must still have many more in store. With the advances in synthetic methodology and the development of more sophisticated isolation and analytical techniques, many more of these phytochemicals should be identified.

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