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Research Article

Evaluation of the Divergent Phytochemicals Contained in Aqueous Leaves Extracts of *Artemisia annua* L. var *chiknensis* and *Cannabis sativa* L. Using HPLC and GC-MS Analytical Protocols

Ejembi Patricia E[1]*, Chike IC Ogbonna[1], Ogbonna Abigail I[1], James O Ejembi[2] and Olukose J Shola[2]

[1]*Applied Microbiology Unit, Department of Plant Science and Biotechnology, University of Jos, Nigeria*

[2]*Department of General Surgery, Federal Medical Center Birnin Kebbi, Kebbi State, Nigeria*

[3]*Department of Laboratory Services, Faith Alive Foundation, Jos, Plateau State, Nigeria*

***Corresponding Author:** Ejembi Patricia E, Applied Microbiology Unit, Department of Plant Science and Biotechnology, University of Jos, Nigeria.

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Abstract

Objective: To investigate the biochemical constituents and antioxidant status of aqueous leaves extracts of *Artemisia annua* and *Cannabis sativa.*

Methods: Phytochemical screening and antioxidant assay of the crude extracts of experimental plants was carried out to identify the various bioactive constituents and the antioxidants scavenging activities of extracts. Furthermore, the phytocomplex components present in the crude aqueous extracts were analyzed using high performance liquid chromatography (HPLC), gas chromatography - mass spectroscopy (GC.MS) and 1,1-diphenyl-2-picrylhydrazyl hydrate (DPPH) radical scavenging activities respectively.

Results: The High Performance Liquid Chromatography (HPLC) of aqueous leaf extract of *Artemisia annua,* revealed the presence of artemisinic acid (2.446), dihydroartemisinin (3.965), artemisinin (6.171) and deoxartemisinin (7.808) while Gas Chromatography-Mass Spectroscopy (GC-MS) revealed the presence of 43 constituents with Deoxyquinghaosu (8.46%), Benzene (5.15%), 4,8-Dihydroximino-4 (3.38%), Scopoletin (3.66%) and Coumarin (1.01%) as the major constituents. Similarly, cannabidiol (CBD) (3.461), cannabidiolic acid (CBDA) (3.620) and Delta-9 Tetrahydro Cannabinol (THC) (6.598) were detected in the aqueous leaf extract of *Cannabis sativa*, through HPLC while the results from GC-MS of aqueous leaf extract of *Cannabis sativa* revealed 29 biochemical constituents with the following as the major ones; Butanoic acid (6.27%), Cyclopentasiloxane (4.03%), Cyclohexasiloxane (5.11%), Cycloheptasiloxane (9.54%), Cyclooctasiloxane (5.21%), Decahydronaphtho [2,3-b]furan-2-one (6.75%), Trimethyl[4-(1-methyl-1-methoxyethyl) phenoxy]silane (4.65%), Arsenous acid (4.37%) and Acetamide (5.89%) but Cannabiniods were not detected. Additionally, the aqueous leaf extract of *A. annua* showed a higher antioxidant activity compared with *C. sativa.*

Conclusion: Many of these phytocomponents have antidiabetic, antitumor, antihypertensive, heart rate reducing, antibacterial, antifungal, antiviral, hepatoprotective, immunomodulatory activities which justify why both experimental plants are employed in alleviating human ailments and other industrial purposes.

Keywords: *Artemisia annua*; *Cannabis sativa;* Phytocomponents; Antioxidant Activity

Introduction

In traditional practice, plant leaves and sometimes the whole plant have been used due to their therapeutic potential for the management and prevention of malaria, diabetes, cancer, epilepsy, psychoneurosis, depression, insomnia, anxiety, irritability and stress [1]. *Artemisia annua* and *Cannabis sativa* are both traditional herbs employed in Nigeria for the management of diseases and belong to families, Asteraceae and Cannabaceae respectively [2,3]. It has been reported that more than 500 species of *Artemisia annua* [4] and 4 species of *Cannabis sativa* [5], are distributed around the globe. The health benefits of *Artemisia annua*, is centered in the content of its artemisinin and the other highly valued constituents which has drawn a huge attention of the scientific community. More, it shows antispasmotic, antiseptic, antibacterial, antimalarial, antitumor, antirheumatic, antidiabetic and antiviral hepatoprotective activities [6,7]. Further, analgesic and anti-inflammatory activities of the *Artemisia annua* were supposed to be due to the presence of artemisinin. Hence, continuous identification of the value-added compounds are very important, scientifically and commercially. The primary constituents found in *C. sativa* are the cannabinoids which are terpenophenolics comprising a diphenol and a monoterpene moiety and other non-cannabinoids such as flavonoids. *Cannabis* species are used to provide relief for tumor-associated symptoms including nausea, anorexia, and neuropathic pain and may decelerate tumor progression in breast cancer patients [8]. Further, *Cannabis sativa* is used in epilepsy, tetanus, rheumatism and cholera [9] and other clinical and industrial activities. Both species of *Artemisia annua* and *Cannabis sativa* are rich in flavonoids. Flavonoids have been known for their antioxidant activities linked to numerous health benefits [10].

Materials and Methods

Collection and preparation of plant materials

The study protocol was approved by the ethical committee of University of Jos for laboratory animal care and experimentation with the reference number: F17-00379. The leaves of *Artemisia annua* was collected from Artemisia plantation in Rayfield Jos, Nigeria, identified and authenticated by Professor C.I.C. Ogbonna of the Department of Plant Science and Biotechnology University of Jos and a voucher specimen (CBGE/CHNA/09/LTNGS/G) was issued while the leaves of *Cannabis sativa* was supplied by National Drug Law Enforcement Agency (NDLEA) Jos command. The leaves were air-dried at room temperature, milled to powder with the aid of an improvised blender.

Phytochemical assessment

The phytochemical screening was carried out on the leaf extracts of test plants using simple standard procedures as described by [11], while the quantitative phytochemical analysis was done using High performance liquid chromatography (HPLC) and Gas chromatography- mass spectroscopy (GC-MS) procedures. Identification of the relative quantity of the chemical compounds present in each of the aqueous leaf extracts of *A. annua* and *Cannabis sativa* was carried out by comparing the mass spectra obtained in the chromatograph with those of the standard mass spectra from National Institute of Standard and Technology library (NIST II) attached to the HPLC and GC-MS instrument.

Antioxidant capacity evaluation

The free radical scavenging activity of *Artemisia annua* and *Cannabis sativa* aqueous extracts were measured in terms of the hydrogen donating or radical scavenging ability using the DPPH. The scavenging activity of the extracts against DPPH radical was determined according to the method of [12]. 500 µl of 0.11 mM methanolic DPPH was added to 500µl of different concentrations (2.5 - 40 mg/ml) of the extracts in test tubes and butylated hydroxytoluene (BHT) and incubated at room temperature in the dark for 10 minutes. The absorbance of the blank (Ab) and samples (As) was measured at 517nm in a spectrophotometer. A control was prepared as above without the sample and distilled water was used for base line correction [13].

Data analysis

The IC_{50} values for DPPH inhibition were determined by fitting the percentage inhibitions calculated from absorbance data to a sigmoidal dose response curve using Origin 7.0 software.

Results

Qualitative screening

Results presented in table 1 shows the phytochemical screening of *A.annua* and *C. sativa*. A variety of bioactive constituents were revealed and they included; Alkaloids, Saponins, Tannins, Flavonoids, Terpenes, Carbohydrates and Cardiac glycoside. The presence of these chemical substances suggests that *A. annua* and *C. sativa* could be useful in the prevention and treatment of different ailments in humans as well as in the industrial settings*.

Biochemical constituents	*A. annua*	*C. sativa*
Alkaloids	+++	+++
Saponins	++	-
Tannins	+++	+
Flavonoids	+++	++
Carbohydrates	++	+
Steroids	-	-
Terpenes	++	-
Anthroquinoes	-	-
Cardiac glycoside	+	+

Table 1: Phytochemical Analysis of Aqueous Leaf Extracts of *Artemisia annua* and *Cannabis sativa.*

+ = Present, – = Not present.

High performance liquid chromatography (HPLC) analysis of aqueous leaf extracts of *Artemisia annua* and *Cannabis sativa*

Employing the use of HPLC in the quantitative analysis, artemisinic acid (2.446), dihydroartemisinin (3.965), artemisinin(6.171) and deoxyartemisinin (7.808) were detected in the aqueous leaf extract of *Artemisia annua* with UV traces at different peaks and retention times on the HPLC (Table 2). Similarly, cannabidiol (CBD), (3.461), cannabidiolic acid (CBDA) (3.620) and delta-9 tetrahydrocannabinol (THC) (6.598) were detected in the aqueous leaf extract of *Cannabis sativa* (Table 3).

Gas chromatography - mass spectroscopy (GC-MS) analysis of aqueous leaf extracts of *Artemisia annua* and *Cannabis sativa*

The GC-MS analysis of aqueous leaf extract of *Artemisia annua* revealed the presence of 43 constituents which mostly belong to monoterpene, sesquiterpene, and phenolic acids. The major ones are; Deoxyquinghaosu (8.46%), Benzene (5.15%), 4,8-Dihydroxyimin-o-4(3.86), Scopoletin (3.66%) and Coumarin (1.01%), (Table 4). The compounds were identified based on the fragmentation pattern of mass spectra. The aqueous leaf extracts of *Cannabis sativa* showed the presence of 29 biochemical compounds with the following as the major constituents; Butanoic acid (6.27%), Cyclopentasiloxane (4.03%), Cyclohexasiloxane (5.11%), Cycloheptasiloxane (9.54%), Cyclooctasiloxane (5.21%), Decahydronaphtho[2,3-b]furan-2-one (6.75%), Trimethyl[4-(1-methyl-1-methoxyethyl)phenoxy]silane (4.65%), Arsenous acid (4.37%) and Acetamide (5.89%) (Table 5).

Peak#	Retention time Aqueous A.a	Compound
1	1.980	Solvent
2	2.446	Artemisinic acid
3	3.965	dihydroartemisini
4	6.171	Artemisinin
5	7.808	deoxartemisinin

Table 2: Peaks and Retention Times for HPLC-UV Analysis of Artemisinins in Aqueous Leaf Extract of *Artemisia annua* L.

A.a – *Artemisia annua.*

Peak#	Retention time	Compound
1	-	-
2	-	-
3	2.248	Solvent
4	3.461	CBD
5	3.620	CBDA
6	6.598	THC

Table 3: Peaks and Retention Times for Cannabinoids in Aqueous Leaf Extract of *Cannabis sativa* L.

CBD = Cannabidiol, CBDA = Cannabidiolic Acid, THC- = Tetrahydrocannabinol.

Peak	Retention Time	Area %	Compounds	Class
1	2.510	0.03	Cyclotrisiloxane	Siloxane
2	2.651	0.09	Arsenous acid	Inorganic acid
3	4.341	0.05	Tris(tert-butyldimethylsilyloxy)arsane	
4	4.398	0.06	1,1,1,3,5,5,5-Heptamethyltrisiloxane	Siloxane
5	4.905	0.39	1,3,5,7-Cyclooctatetraene	Alkene
6	5.327	0.14	Cyclotetrasiloxane	Siloxane
7	6.961	0.05	1,1,1,3,5,5,5-Heptamethyltrisiloxane	Siloxane
8	7.355	0.04	1,4-Bis(trimethylsilyl)benzene	Benzene derivative
9	7.468	0.11	1,2-Bis(trimethylsilyl)benzene	Benzene derivative

10	7.722	0.04	2,4,6-Cycloheptatrien-1-one	Alkanone
11	8.510	1.22	Cyclopentasiloxane	Siloxane
12	8.736	0.12	Benzo[h]quinoline	Quinoline
13	9.017	0.05	3,3-Diisopropoxy-1,1,1,5,5,5-hexam ethyltrisiloxane	Siloxane derivative
14	9.186	0.04	[1,2,4]Triazolo[1,5-a]pyrimidine-6 carboxylic acid	Alkanolic acid
15	9.327	0.10	Benzo[h]quinoline	Quinoline
16	9.496	0.05	Octasiloxane	Siloxane
17	10.144	0.32	4-Bromo-3-chloroaniline	Aniline derivative
18	10.285	1.47	Cyclohexasiloxane	Siloxane
19	10.961	1.28	.alpha.-D-Ribofuranoside	
20	11.384	1.01	Coumarin	Coumarin
21	11.609	1.85	Cycloheptasiloxane	Siloxane
22	11.947	0.38	Anthracene	Anthracene
23	12.313	1.15	2-Ethylacridine	
24	12.680	1.02	Cycooctasiloxane	Siloxane
25	12.792	0.97	5,5'-Di(ethoxycarbonyl)-3	
26	13.018	1.58	trans-3-Ethoxy-b-methyl-b-nitrosty rene	Alkene
27	13.187	1.11	1H-Indole	Indole
28	13.356	5.15	Benzene	Aromatic hydrocarbon
29	13.553	3.73	Bicyclo[2.2.2]octane	Alkane
30	13.778	1.78	Cyclohexane-1	Alkane
31	13.947	2.93	Bicyclo[4.1.0]hepta-2,4-diene	Alkene
32	14.144	2.10	4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine	
33	14.539	3.66	5,6-Dimethoxy-1-indanone, Scopoletin	Coumarin
34	14.680	2.45	Bicyclo[3.3.1]nonan-2-one	
35	14.849	2.43	4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine	
36	14.933	1.79	2,4,6-Trimethylphenyl isothiocyanate	
37	15.074	8.46	Deoxyqinghaosu	Sesquinterpenes
38	15.412	2.58	Fluorenone	Alkanone
39	15.609	2.66	N-(4-Oxotricyclo[3.3.1.1[3,7]]dec-2-yl)acetamide	Amide
40	15.750	2.80	1H-Purin-2-amine	Amine
41	16.257	3.83	4,8-Dihydroxyimino-4	
42	16.426	2.02	1,2-Benzisothiazol-3-amine tms	Amine
42	18.229	1.49	Tetrasiloxane	Siloxane
43	18.595	3.59	Benzonitrile	Nitrile

Table 4: Quantitative Analysis of Aqueous Leaf Extract of *Artemisia annua* by GC-MS.

Peak	Retention Time	Area %	Compounds	Class
1	12.698	1.22	Propanoic acid	Carboxylic acid
2	3.299	0.94	Cyclotrisiloxane	Siloxane
3	3.637	6.27	Butanoic acid	Carboxylic acid
4	3.834	1.33	Benzenepropanoic acid	Carboxylic acid
5	4.313	0.79	Hexanoic acid	Carboxylic acid
6	8.567	4.03	Cyclopentasiloxane	Organosilicon
7	10.285	5.11	Cyclohexasiloxane	Organosilicon
8	11.609	9.54	Cycloheptasiloxane	Organosilicon
9	12.229	1.43	1,4-Bis(trimethylsilyl)benzene	Organosilicon
10	12.680	5.21	Cyclooctasiloxane	Organosilicon
11	12.764	3.03	Benzene	Aromatic hydrocarbon
12	13.018	1.53	Trimethyl[4-(1,1,3,3,-tetramethylb utyl)phenoxy]silane	Organosilicon
13	13.299	1.81	1,2-Bis(trimethylsilyl)benzene	Organosilicon
14	13.778	3.83	Indole-2-one	Ketone
15	13.947	6.75	Decahydronaphtho[2,3-b]furan-2-one	Ketone
16	14.116	4.65	Trimethyl[4-(1-methyl-1-methoxyeth yl)phenoxy]silane	Organosilicon
17	14.398	5.03	Methyltris(trimethylsiloxy)silane	Organosilicon
18	14.680	4.37	Arsenous acid	Inorganic acid
19	15.299	3.91	Heptasiloxane	Organosilicon
20	16.398	1.88	Cyclotrisiloxane	Organosilicon
21	17.018	5.89	Acetamide, octyl ester	Ester
22	17.215	3.87	Tris(tert-butyldimethylsilyloxy)arsane	Arsa alkenes
23	17.581	2.18	Trimethyl[4-(2-methyl-4-oxo-2-pent yl)phenoxy]silane	Organosilicon
24	18.201	1.04	Methyltris(trimethylsiloxy)silane	Organosilicon

Table 5: Quantitative Analysis of Aqueous Leaf Extract of *Cannabis sativa* L using GC-MS.

Antioxidant activity

Antioxidant properties of aqueous leaf extracts of *A. annua* and *C. sativa* were measured using DPPH radical scavenging activity. The crude aqueous extract of *A. annua* was found to be effective in scavenging the DPPH radicals. The DPPH radical scavenging properties were found to be concentration dependent. The greater scavenging capacity means the higher antioxidant activity. Similarly, IC_{50} values of aqueous extracts of *A. annua* and *C. sativa* were found to have 2.86 ± 0.65 mg/mL and 4.12 ± 0.70 mg/mL, respectively whereas ascorbic acid was 3.44 ± 0.88^{ab} (Table 6).

% DPPH scavenging activities			
Conc. (mg/ml)	***A. annua***	***C. sativa***	**Vitamin C**
2.5	31.69 ± 2.57	31.88 ± 1.54	26.46 ± 2.10
5	32.56 ± 0.71	32.69 ± 0.77	27.14 ± 3.00
10	32.67 ± 0.48	32.19 ± 0.38	33.85 ± 0.82
20	33.56 ± 0.61	33.52 ± 0.31	33.80 ± 1.40
40	36.13 ± 0.81	33.95 ± 0.68	34.85 ± 0.18
IC50	2.86 ± 0.65^{a}	4.12 ± 0.70^{b}	3.44 ± 0.88^{ab}

Table 6: 1,1-Diphenyl-2-picrylhydrazyl hydrate (DPPH) Radical Scavenging Activity of Aqueous Leaf Extracts of *Artemisia annua* and *Cannabis sativa.*

Values are mean ± standard deviation of triplicate determinations.

Discussion

The medicinal and biological properties of the phyto-compounds identified in the aqueous leaves extracts of *A. annua* and *C. sativa* have activities like antibacterial, antifungal, antiviral, antidiabetic, and antitumor and immunomodulatory activities. The presence of such phytonutrients ensures the medicinal uses of these plant species. Among these phytonutrients flavonoids and alkaloids have gained significant scientific interest due to their reducing properties. Flavonoids are a diverse group of phytonutrients that are produced by various plants species. Based on their skeleton, flavonoids are classified into eight groups: flavans, flavanones, isoflavanones, flavones, isoflavones, anthocyanidines, chalcones and flavonolignans. Flavonoids play important roles in plant growth and development, and in defense of plants against microorganisms and pests serving as means of plant-animal warfare. The best-described property of almost every group of flavonoids has been their capacity to act as antioxidants. The flavonoids seem to be the most powerful for protecting the body against reactive oxygen species. Alkaloids on the other hand rank among the most efficient and therapeutically significant plant substances [14]. Pure, isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodiac and bactericidal effects [15]. The genus, *Artemisia annua* produced artemisinin which is a potential source for the treatment of a broad array of ailments.

Artemisinin an endoperoxide sesquiterpene lactone with the common name "qinghaosu", isolated from the Chinese medicinal plant *A. annua*, has provided a new class of highly effective antimalarials. Artemisinin-based combination therapies are now generally considered as the best current treatment for uncomplicated *Plasmodium falciparum* malaria [16]. From our work, using UV- HPLC analysis revealed 6.171% artemisinin content which is in agreement with the finding of [17] who reported 7.3% but higher than the 4.8% and 3.46 reported by [7,18] respectively. Other constituents of *Artemisia annua* include deoxyartemisinin, artemisinic acid, arteannuin- B, as also reported by [7]. However, the analysis revealed that the constituents of aqueous leaf extract of *A. annua* mostly belong to monoterpene, sesquiterpene, and phenolic acids. From our research, the biochemical components revealed in GC-MS are 43 and the major ones are; Deoxyquinghaosu (8.46%), Benzene (5.15%), 4,8-Dihydroxyimin-o-4(3.86), Scopoletin (3.66%) and Coumarin (1.01%). Coumarins are natural substances derived from benzo-a-pyrone with one or more phenolic functions were present in the aqueous leaf extract of *A. annua*. Coumarins; scopoletine, possess immune-modulatory properties that are mostly suppressive [19]. Coumarins and its derivatives draw scientific attention due to their photochemotherapy and therapeutic application in cancer. The percentage of artemisinin (8.46%), presents in the extract of *Artemisia annua* as revealed by the analytical tool; GC-MS in our study is the highest so far around the globe as no such quantity has been reported before now. The antioxidant capacity of the test plant is associated with the high content of flavonoids and the diversity of other compounds [20].

Phytocannabiniods: comprise of over 500 compounds in the plant *C. sativa* making it a complex matrix. Cannabinoids are mostly biosynthesized in an acidic form, among which the most abundant is cannabidiolic acid (CBDA) and Tetrahydro Cannabinolic Acid (THCA). However, these acidic forms are not stable as they may decompose in the presence of heat or light. Acidic cannabiniods are therefore decarboxylated to their neutral homoloques, as in the case of THCA to THC [21]. THC is accepted to be the main psycoactive agent as it possesses analgesic, anti-inflammatory, appetite stimulant, and antiemetic properties; it can also protect the brain from cognitive deficit at very low doses [22]. However, continuous use of the plant may cause cognitive deficits at least in adolescents, since until the early 30s they have significant neurodevelopmental changes. The neurocognitive effects of extended use in adults are somewhat inconsistent [23]. Cannabidiol can modulate euphoric effects of THC and has antipsychotic, neuroprotective, anticancer, antidiabetic, and other effects such as reducing the anxiety induced by cigarette consumption in tobacco smokers [24].

The results for the quantitative measurement of cannabinoids by HPLC-UV method revealed Cannabidiol (CBD), (3.461), cannabidiolic acid (CBDA) (3.620) and delta-9 tetrahydrocannabinol (THC) (6.598) were detected in the aqueous leaf extract of *C. sativa*. The components found in the plant are in agreement with the other works of Wang [25] and Zivovinovic [26]. Based on the GC-MS analysis of the aqueous extract of *C. sativa* only non-cannabiniods were detected which were mostly carboxylic acids, organosilicon and ketones.

The detection of certain bioactive constituents in the aqueous leaf extracts of *Artemisia annua* and *Cannabis sativa* are completely new as no such work has ever been carried out.

Conclusion

Both *A. annua* and *C. sativa* possessed very high levels of alkaloids and flavonoids and are employed in medicinal uses. The plants investigated can be seen as a potential source of useful drugs. Further, both plants showed good antioxidant property which informed the use of leaves of the plants for different human diseases by traditional practitioners. The present study ascertain the potentials of *A. annua* and *C. sativa*, their chemical compositions and biological properties of the aqueous or alcoholic extracts can vary considerably depending on their geographical origin, the plant materials used and the way it is treated. GC-MS analysis of *A. annua* unveiled artemisinin (8.46%) and scopoletin (3.66%) which are the highest in terms of concentrations in this plant so far around the globe. It should be mentioned here that the scientific society should not allow such a high impact discovery fall into oblivion as such might be a catastrophic error.

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Conflicts of Interests

The authors also wish to declare that there was no conflict of interest/competing interest among them.

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