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Screening for Pharmacological Compounds and Antioxidant Activity of *Hedychium coronarium* J. Koenig

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Authors' contributions

This work was carried out in collaboration among all authors. Authors EAS and NCN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EAS, WGP and GBO managed the analyses of the study. Authors EAS, NCN and GBO managed the literature searches. All authors read and approved the final manuscript

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ABSTRACT

Screening for pharmacological compounds and antioxidants activity of *Hedychium coronarium* leaf sample was undertaking. Phytochemicals, gas chromatography-mass spectrometry (GC-MS) analysis and antioxidant activity of the leaf sample were evaluated using standard methods. The result of phytochemical screening revealed the presence of alkaloids, flavonoids, cardiac glycosides, phlobatannins, and proteins. The GC-MS analysis of the leaf extract revealed the presence of indole, phytol and other important compounds of interest. The leaf extract also exhibited better radical scavenging activity against the control in the present study. The bulk of the phytochemicals found present and some of the compounds revealed by GC-MS analysis which are biologically active with physiological activities, could be behind the antioxidant activity of the leaf sample. They could as well be responsible for the pharmacological benefits derived from the leaf of *H. coronarium.* This study has revealed the pharmacological compounds and antioxidant activity of *H. coronarium* leaf sample.

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1. INTRODUCTION

Plants have been of immense importance to humans as well as other creatures [1-10]. Humans use plants in many numerous ways because of their importance [11-14]. Plants have been a major source of food to humans [15-22] and most of these foods are healthy [23-30]. Foods of plant origin nourish the body of organisms when eaten, digested and assimilated [14-21,22,24]. Apart from the function of provision of food substances [5,8,13-22,24-31], plants with their intent medicinal activities have salvaged many diseases through the practice of traditional medicine or herbalism [32-35]. Tiburt and Kaptchuk [36] described traditional herbal medicine as naturally occurring plant-derived substances with minimal or no industrial processing that has been used to treat illness within local or regional healing practices. Different authors have also described such practice with different names such as folk medicine, indigenous medicine, or botanical medicine [32-35,37]. Plants with intent medicinal activities and are employed in traditional medicine practice against diseases, are known as medicinal plants. Sofowora et al. [38] and WHO [39] defined a medicinal plant as any plant which in one or more of its organs contain substances that can be employed for therapeutic purposes or which are precursors for the synthesis of useful drugs. Another school of thought also defined medicinal plants as those plants that possess therapeutic properties or exert beneficial pharmacological effect on the human or animal body. According to World Health Organization, about 80% of the world population depends on herbal medicine [40]. Amadi et al. [28] noted that efficacy of medicinal plants against ill health is possible due to certain numerous biologically active compounds. Different authors have identified those biologically active compounds as nutrients, phytochemicals, vitamins, and minerals [41-45].

Antioxidant activity is among the wide range of biologically and pharmacological activities exhibited by medicinal plants. Antioxidants are substances or compounds that slow the work of oxidative stress inducing molecules in the body. These stress inducing molecules are biologically termed reactive oxygen species (ROS) [46-48]. When the complex of antioxidant systems such as major cellular redox buffers ascorbate (AsA), glutathione (γ-glutamyl-cysteinyl-glycine, GSH), tocopherol, carotenoids, phenolic compounds; and enzymatic components such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), enzymes of ascorbate-glutathione (AsA-GSH) cycle, ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) are overpowered by these reactive oxygen species, oxidative stress would set in [49-53].

Hedychium coronarium J. Koenig popularly known as “white ginger” is one of the invasion plants of the planet Earth [54]. It is a perennial herbaceous plant which belongs to Zingiberaceae family. The plant is believed to have originated from Tropical Asia and the Himalayas [54]. *H .coronarium* grows up to 1 to 2.5 m tall and produces a thick mat creeping stems that run underground as rhizomes close to the soil surface [54]. The rhizomes are well edible and have medicinal properties [54-55]. According to Chatham et al. [55] and Lemino Singh et al. [56], the Zingiberaceae plant *H. coronarium* has many common names such as butterfly lily, butterfly ginger, garland flower, ginger lily and cinnamon jasmine. The plant needs a well drained moist soil with lots of organic matter to survive. Extract of the rhizomes of *H. coronarium* has been implicated in the treatment of internal injuries, liver complains, thickening of blood, bronchitis and asthma. Powder form of the rhizomes is antiseptic in nature. It is used against various pains and aches as poultice. Decoction of rhizomes is effective for mouth wash against bad breath. Paste made from rhizomes is effective against bruises and sprains [54]. These benefits derivable from *H. coronarium* could be from those numerous biologically active compounds that exhibit physiological action when they come intact with target tissue or organ of the body.

Since natural compounds found in plants or their synthetic forms are the basis of modern pharmacopeia [57-58], the present study tried to unravel more possible pharmacological compounds from *H. coronarium* through screening for bioactive compounds using advanced technique and evaluated the antioxidant activity of the plant using the leaf sample.

2. MATERIALS AND METHODS

2.1 Sample Collection and Identification

The *H. coronarium* plant used in this study was harvested from a farm in Umunchi village, Isiala Mbano LGA of Imo State, Nigeria. The plant was properly identified by a Botanist in the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria as *H. coronarium*. A voucher specimen (RSU/DMS/20/018) was deposited at the herbarium unit of Rivers State University. The leaves of the plant were harvested, cleaned and air dried, before they were milled into powder for further studies.

2.2 Preparation of Aqueous Extract

The aqueous extract was prepared with twenty gram of the milled sample of *H. coronarium* using the method as described by Ezekwe et al. [59]. The prepared extract was used for some of the qualitative phytochemical studies, GC-MS analysis and evaluation of antioxidant activity.

2.3 Qualitative Phytochemical Determinations

2.3.1 Test for alkaloids

One (1) mL each of the extract was shaken with 5 mL of 2% HCl on a steam bath and then filtered. To I mL of the filtrate, Wagner`s reagent (iodine in potassium –iodide solution) was added and reddish brown precipitates was observed for positive result.

2.3.2 Test for anthroquinones

One gram of the seed extract was placed in a dry test tube and 20 mL of chloroform was added. This was heated in steam bath for 5 min. The extract was filtered while hot and allowed to cool. To the filtrate was added with an equal volume of 10% ammonia solution. This was shaken and the upper aqueous layer was observed for bright pink colouration, which for the presence of anthraquinones. This was repeated with all the plant samples.

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2.3.3 Test for flavonoids

0.5 g of the extract was added, in a test tube and 10 ml of distilled water, 5 mL of dilute ammonia solution were added to a portion of the aqueous filtrate of the extract followed by addition of 1 mL concentrated H_2S0_4. Indication of yellow color shows the presence of flavonoid in each extract.

2.3.4 Cardiac glycosides

One mL of the seed extract was dissolved in 2 mL of chloroform in a test tube. 1 mL conc. H_2SO_4 was carefully added to the test tubes through the side and was observed for a red or reddish brown colouration at the interphase, which indicates positive result.

2.3.5 Test for phlobatannins

One percent aqueous hydrochloric acid was added to the seed extract in a test tube (about 2 mL), and then boiled with the help of Hot plate stirrer. Formation of red coloured precipitate confirmed a positive result.

2.3.6 Test for phenolic compounds

To 2 mL of the seed extract, 1% $FeCl_3$ was added and observation was made for blue, violet, purple, green or red-brown colour.

2.3.6 Test for proteins

Five drops of 1% hydrated copper sulphate was added to 2 mL the seed extract in a test tubes. Two mL of 40% NaOH was also added, and the test tube was shaken vigorously to mix the content and presence of purple colouration indicated the presence of proteins..

2.3.7 Test for reducing sugars

One mL of ethanol was mixed with 2 mL each of the plant extract, after which 1 mL each of Fehling solution A and B were added to the test tubes. The test tubes were heated to boiling while observation was made for presence of reddish brown colouration which indicates positive results.

2.3.8 Test for tannins

To 1 mL of the extract, equal volume of bromine water was added. The formation of a greenish to red precipitate was taken as the presence of tannins.

2.3.9 Test for saponins

One mL of the extract was boiled with 5 mL of distilled water for 5 min. and decanted while hot.

4 mL of distilled water was added to 1 mL of the filtrate before it was shaken vigorously for observation of stable froth on standing.

2.3.10 Test for steroid

Half (0.5 g) gram of the extract was dissolved in 10 mL anhydrous chloroform and filtered. The filtrate was divided into two equal portions for the following tests. The first portion of the solution above was mixed with one mL of acetic anhydride followed by the addition of 1 mL of concentrated sulphuric acid down the side of the test tube to form a layer underneath. The test tube was observed for green colouration as indicative of steroids.

2.3.11 Test for terpenoids

One gram of seed sample was shaken in a test tube with 10 mL of methanol, and then filtered. 5 mL extract was then mixed with 2 mL of chloroform and 3 mL of sulphuric acid was added. Formation of reddish brown color indicates the presence of terpenoids in the selected plants.

2.4 GC-MS Analysis of the Extracts

The method described by Ezekwe et al. [57] was used for GC-MS analysis. GC-MS analysis of the aqueous extracts was carried out using AOC-20i auto sampler and gas chromatograph interface to a mass spectrometer (GC-MS) instrument. Employing the following conditions; column Elite-1 fused silica capillary column (30 mm×0.25 mm ID×1Mm df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 Ev; helium (99.999%) was used as carrier gas at a constant flow of 1 ml/ min, and an injection volume of 0.5μl, Split ratio of 10:1), with injector temperature 250°C; and ion-source temperature 280°C. The oven temperature was programmed from 110°C (Isothermal for 2 min), with an increase of 10°C/min to 200°C, then 5°C/min to 280°C, ending with a 9 mins isothermal at 280°C. Mass spectra were taken at 70 Ev; a scan interval of 0.5 seconds and fragments from 45 to 450Da. Total GC running time was 36 mins. The plant extract was dissolved in aqueous and filtered with polymeric solid phase extraction (SPE) column and analyzed in GC-MS for different components. Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62 000 patterns. The spectrum of the unknown components was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test were ascertained.

2.5 Determination of Antioxidant Activity

2.5.1 DPPH (1, 1-Dipheny l-2-picrylhydrazyl) radical scavenging assay

The free radical scavenging activity was measured by DPPH assay method. Four mg of DPPH (0.1 Mm) was dissolved in 100 Ml of distill water to obtain working solution. One Ml of each extract was mixed separately with 2.0 Ml of 0.1 Mm DPPH followed by 30 min incubation in dark. The reduction of the DPPH free radical was measured by taking the absorbance at 517 nm [60]. Colour of DPPH was reduced from purple to yellow. The antioxidant activity of each extracts was evaluated by calculating the inhibition % of free radical formation using the formula:

% inhibition = [(A-A1)/A] x 100; A=absorbance of the blank (DPPH); A1=absorbance of the extract (DPPH+ extract).

3. RESULTS AND DISCUSSION

Table of phytochemical screening of *H. coronarium* (Table 1) shows that twelve phytochemicals namely alkaloids, anthraquinnones, flavonoids, cardiac glycosides, phlobactanins, phenolic compounds, proteins, reducing sugar, tannins, saponins, and terpernoids were screened, out of which only alkaloids, flavonoids, cardiac glycosides, phlobactanins, proteins, tannins, saponins and terpenoids were found present. Alkaloids, phobactanins, tannins, and saponins were in highly concentrated while flavonoids, cardiac glycosides, proteins, and terpenoids were moderate in concentrations. These phytochemicals are important bioactive constituents behind plants physiological action and pharmacological importance. Isolated plant alkaloids and their synthetic derivatives are used as basic medical agents for analgesic and bactericidal effect [2,61]. Flavonoids scavenge hydroxyl radicals, superoxide anion, and lipid proxy radicals [2-3,33,42,62]. Cardiac glycoside has been associated with proper heart function [3,62]. Steroids share a relationship with all steroid hormones [62]. Phlobatannins have

wound healing ability, analgesic, anti-inflammatory and antioxidant properties [63]. Tannins have astringent taste, hasten the healing of wounds and inflamed mucous membranes [2]. The role of protein in the replacement of worn-out cells and tissues as well as energy production has been noted. Saponins have been associated with better taste and antibacterial properties. The inhibitory property of terpenoids against cholesterol has been reported [63]. Also, the anti-viral, anti-malarial, anti-inflammatory, and anti-bacterial properties of terpenoids have been reported by wadood et al. [63].

Apart from the bucket phytochemicals found in *H. coronarium* the leaf sample. Further studies using a more detailed analytical technique GC-MS analyzer showed over eighty (80) compounds with their retention time, molecular weights and peaks. These compounds include 2-Ispropyl-1-methoxy-4-methylbenzene, 1,2-Benzenedicarboxylic acid, dicyclohexyl ester, Propanoic acid, 2-(aminooxy)-, Benzofuran, 2,3-dihydro-,1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-, Acetic acid, mercapto-, 1,2-ethanediyl ester, 4-Piperidinone, 1-methyl-, Benzenepropanoic acid, methyl ester, Indole, 4-Hydroxy-3-methylacetophenone, N-(1,1-Dimethylpropynyl)acrilamide, Cyclotrisiloxane, hexamethyl-,2H-Pyran, 5,6-dihydro-2-methyl-,1-Hydroxylinalool, 2,7-Dimethyl-2,7-octanediol, 2-Butenedioic acid, 2,3-dibromo-, (Z)-, Phenol, 4-(2-aminoethyl)-, Bicyclo [3.1.0]hex-3-en-2-one, 4-methyl-1-(1-methylethyl)-, 2H-Indol-2-one, 1,3-dihydro-, Piperazine, 1-[2-[4,5-dichlorophenylethyl]-4-propyl, 5-Methyl-7-acetylamino-S-triazolo [1,5-a]pyrimidine, Tricyclo[4.3.1.13,8]undecane-3-carboxylic acid, Acetophenone, 4'-amino-, Quinoline, 3-methyl-, Indene, 2(1H)-Naphthalenone, octahydro-4,4a-dimethyl-, (4α,4aα,8aβ)-, 3,4-Undecadiene-2,10-dione, 6,6-dimethyl-,1,1,1,3,5,7,7,7-Octamethyltetrasiloxane, Silane, trimethyl [5-methyl-2-(1-methylethyl)phenoxy]-, 2,5-Dimethyl-1-propylpyrrole, Pyridinium, 1-(acetylamino)-, hydroxide, inner salt, 7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl-,2,3-Dioxabicyclo [2.2.2]oct-5-ene, 1-methyl-4-(1-methylethyl)-, 7-Oxabicyclo [4.1.0]heptane, 1-methyl-4-(2-methyloxiranyl)-, 7-Oxabicyclo [4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl-,5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-, 1H-Indole-3-acetic acid, methyl ester, 1,3,2-Dioxaborolane-4,5-dimethanol, 2-ethyl-, diacetate, cis-,6,8-Diazabicyclo [3.2.1]oct-6-ene-6-oxide, 8-hydroxy-1,4,5,7-tetramethyl-,10-Undecynoic acid, methyl ester, Bicyclo [3.1.1] heptane, 2,6,6-trimethyl-,2-Pentadecanone, 6,10,14-trimethyl, 2(1H)-Benzocyclooctenone, decahydro-10a-methyl-, trans-, Bicyclo [3.1.1]heptane, 2,6,6-trimethyl-.9-Hexadecenoic acid, methyl ester, (Z)-, Hexadecanoic acid, methyl ester, Hexadecanoic acid, methyl ester, Oleic Acid, 1-Nonadecanol, 9-Octadecenoic acid, methyl ester, (E)-, Phytol,

Table 1. Phytochemical screening of *H. coronarium*

Phytochemical	*H. coronarium*
Alkaloids	++
Anthraquinnones	-
Flavonoids	+
Cardiac glycosides	+
Phlobatannins	++
Phenolic compounds	-
Proteins	+
Reducing sugars	-
Tannins	++
Saponins	++
Steroids	-
Terpenoids	+

++: present in high concentration; +: moderate concentration; -: absent

Table 2. Result of GC-MS analysis of *H. coronarium* showing retention time, molecular formula, molecular weight and peak area

SN	RT	Component	Formula	MW	%
1	3.696	2-Ispropyl-1-methoxy-4-methylbenzene	$C_{11}H_{16}O$	164	3.04
2	3.804	1,2-Benzenedicarboxylic acid, dicyclohexyl ester	C20H26O4	330	3.68
3	3.894	Propanoic acid, 2-(aminooxy)-	$C_3H_7NO_3$	105	0.28
4	3.992	Benzofuran, 2,3-dihydro-	C8H8O	120	8.48
5	4.082	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-	C7H9NO2	139	1.29
6	4.145	Acetic acid, mercapto-, 1,2-ethanediyl ester	$C_6H_{10}O_4S_2$	210	0.37
7	4.194	4-Piperidinone, 1-methyl-	C6H11NO	113	0.80
8	4.337	Benzenepropanoic acid, methyl ester	C10H12O2	164	0.35
9	4.483	Indole	C_8H_7N	117	0.89
10	4.588	4-Hydroxy-3-methylacetophenone	C9H10O2	150	2.76
11	4.655	N-(1,1-Dimethylpropynyl)acrilamide	$C_8H_{11}NO$	137	0.25
12	4.685	Cyclotrisiloxane, hexamethyl-	C6H18O3Si3	222	0.36
13	4.730	2H-Pyran, 5,6-dihydro-2-methyl-	C6H10O	98	0.30
14	4.839	1-Hydroxylinalool	$C_{10}H_{18}O_2$	170	0.73
15	4.993	2,7-Dimethyl-2,7-octanediol	$C_{10}H_{22}O_2$	174	0.39
16	5.060	2-Butenedioic acid, 2,3-dibromo-, (Z)-	$C_4H_2Br_2O_4$	274	0.35
17	5.158	Phenol, 4-(2-aminoethyl)-	C8H11NO	137	4.56
18	5.304	Bicyclo[3.1.0]hex-3-en-2-one, 4-methyl-1-(1-methylethyl)-	C10H14O	150	0.34
19	5.491	2H-Indol-2-one, 1,3-dihydro-	C8H7NO	133	0.25
20	5.615	Piperazine, 1-[2-[4,5-dichlorophenylethyl]-4-propyl	$C_{15}H_{22}Cl_2N_2$	301	0.31
21	5.671	5-Methyl-7-acetylamino-S-triazolo[1,5-a]pyrimidine	$C_8H_9N_5O$	191	0.3
22	5.716	Tricyclo[4.3.1.13,8]undecane-3-carboxylic acid	$C_{12}H_{18}O_2$	194	0.29
23	5.784	Acetophenone, 4'-amino-	C_8H_9NO	135	0.91
24	5.990	Quinoline, 3-methyl-	$C_{10}H_9N$	143	0.24
25	6.080	Indene	C9H8	116	0.49
26	6.170	2(1H)-Naphthalenone, octahydro-4,4a-dimethyl-, (4α,4aα,8aβ)-	$C_{12}H_{20}O$	180	0.71
27	6.241	3,4-Undecadiene-2,10-dione, 6,6-dimethyl-	$C_{13}H_{20}O_2$	208	0.46
28	6.316	1,1,1,3,5,7,7,7-Octamethyltetrasiloxane	$C_8H_{24}O_3Si_4$	281	0.81
29	6.353	Silane, trimethyl[5-methyl-2-(1-methylethyl)phenoxy]-	$C_{13}H_{22}OSi$	222	0.28
30	6.402	2,5-Dimethyl-1-propylpyrrole	$C_9H_{15}N$	137	0.30
31	6.455	Pyridinium, 1-(acetylamino)-, hydroxide, inner salt	$C_8H_{11}N_2O$	151	1.21
32	6.515	7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl-	$C_{13}H_{22}O_3$	226	2.57

SN	RT	Component	Formula	MW	%
33	6.567	2,3-Dioxabicyclo[2.2.2]oct-5-ene, 1-methyl-4-(1-methylethyl)-	C10H16O2	168	0.36
34	6.691	7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(2-methyloxiranyl)-	C10H16O2	168	0.54
35	6.811	7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl-	C13H22O3	226	0.56
36	6.837	5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-	C13H22O	194	0.26
37	6.961	1H-Indole-3-acetic acid, methyl ester	C11H11NO2	189	0.96
38	7.024	1,3,2-Dioxaborolane-4,5-dimethanol, 2-ethyl-, diacetate, cis-	$C_{10}H_{17}BO_6$	244	1.77
39	7.099	6,8-Diazabicyclo[3.2.1]oct-6-ene-6-oxide, 8-hydroxy-1,4,5,7-tetramethyl-	$C_{10}H_{18}N_2O_2$	198	0.53
40	7.171	10-Undecynoic acid, methyl ester	$C_{12}H_{20}O_2$	196	0.35
41	7.242	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	$C_{10}H_{18}$	138	0.92
42	7.272	2-Pentadecanone, 6,10,14-trimethyl	$C_{18}H_{36}O$	268	0.63
43	7.350	2(1H)-Benzocyclooctenone, decahydro-10a-methyl-, trans-	$C_{13}H_{22}O$	194	0.39
44	7.433	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	$C_{10}H_{18}$	138	0.84
45	7.534	9-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{32}O_2$	268	0.41
46	7.617	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	4.48
47	7.905	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.45
48	8.198	Oleic Acid	$C_{18}H_{34}O_2$	282	0.30
49	8.284	1-Nonadecanol	$C_{19}H_{40}O$	284	1.15z
50	8.363	9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	296	3.95
51	8.423	Phytol	$C_{20}H_{40}O$	296	10.68
52	8.456	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	1.56
53	8.535	iso-C4H9C(O)OCH(CH3)2	$C_8H_{16}O_2$	144	0.49
54	8.588	4,7-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	290	0.32
55	8.655	9,12-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	294	0.40
56	8.715	9,12-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	294	0.79
57	8.771	N-Formyl-3,4-methylenedioxyamphetamine	$C_{11}H_{13}NO_3$	207	0.27
58	8.846	2(1H)-Naphthalenone, octahydro-8a-hydroxy-4a-methyl-, cis-	$C_{11}H_{18}O_2$	182	0.27
59	9.004	Methanethioamide, N,N-dimethyl-	C_3H_7NS	89	0.70
60	9.214	8-Methyloctahydrocoumarin	$C_{10}H_{16}O_2$	168	0.24
61	9.300	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	326	0.59
62	9.465	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	324	0.74
63	9.525	Piperidine	$C_5H_{11}N$	85	0.79
64	9.577	9-Octadecenamide	$C_{18}H_{35}NO$	281	0.29
65	10.166	Nonadecanoic acid, methyl ester	$C_{20}H_{40}O_2$	312	0.85
66	10.301	1,2-Benzenedicarboxylic acid, 3-nitro-	$C_8H_5NO_6$	211	2.02

SN	RT	Component	Formula	MW	%
67	11.189	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	326	0.59
68	11.534	Cyclohexanamine, N-nitro-	$C_{13}H_{22}N_2$	206	0.25
69	11.841	ψ,ψ-Carotene, 7,7',8,8',11,11',12,12',15,15'-decahydro-	$C_{40}H_{66}$	546	1.19
70	12.707	2(1H)-Pyridinone, 1,4,6-trimethyl-	$C_8H_{11}NO$	137	2.38
71	13.42	Imidazo(1,2-a)pyrimidine, 6-methyl-5-oxo-1,2,3,5-tetrahydro-	$C_7H_9N_3O$	151	3.36
72	13.543	9-Borabicyclo[3.3.1]nonan-9-amine, N-methyl-	$C_9H_{18}BN$	151	0.93
73	14.24	3,6-Dimethyl-5-oxo-1,2,3,5-tetrahydroimidazo[1,2-a]pyrimidine	$C_8H_{11}N_3O$	165	5.45
74	15.193	Caryophyllene	$C_{15}H_{24}$	204	1.53
75	15.283	4-Cyclohexene-1,2-dicarboximide, N-butyl-, cis-	$C_{12}H_{17}NO_2$	207	0.38
76	15.493	Chola-5,22-dien-3-ol, (3β,22Z)-	$C_{24}H_{38}O$	342	1.81
77	15.774	Androst-5-en-3-ol, 4,4-dimethyl-, (3β)-	$C_{21}H_{34}O$	302	0.54
78	15.822	4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine	$C_{16}H_{14}N_2O_4$	298	0.48
79	16.047	Kauren-18-ol, acetate, (4β)-	$C_{22}H_{34}O_2$	330	1.51
80	16.43	N-Cyano-N',N',N'',N''-tetramethyl-N-methoxymethyl-1,3,5-triazine-	$C_{10}H_{17}N_7O$	251	1.31
81	16.835	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	222	0.34

Octadecanoic acid, methyl ester, iso-C4H9C(O)OCH(CH3)2, 4,7-Octadecadiynoic acid, methyl ester, 9,12-Octadecadienoic acid, methyl ester, (E,E)-, 9,12-Octadecadienoic acid, methyl ester, (E,E)-, N-Formyl-3,4-methylenedioxyamphetamine, 2(1H)-Naphthalenone, octahydro-8a-hydroxy-4a-methyl-, cis-, Methanethioamide, N,N-dimethyl-,8-Methyloctahydrocoumarin, Eicosanoic acid, methyl ester, 4,8,12,16-Tetramethylheptadecan-4-olide, Piperidine, 9-Octadecenamide, Nonadecanoic acid, methyl ester, 1,2-Benzenedicarboxylic acid, 3-nitro-, Eicosanoic acid, methyl ester, Cyclohexanamine, N-nitro-, ψ,ψ-Carotene, 7,7',8,8',11,11',12,12',15,15'-decahydro-,2(1H)-Pyridinone, 1,4,6-trimethyl-, Imidazo(1,2-a)pyrimidine, 6-methyl-5-oxo-1,2,3,5-tetrahydro-,9-Borabicyclo[3.3.1]nonan-9-amine, N-methyl-,3,6-Dimethyl-5-oxo-1,2,3,5-tetrahydroimidazo [1, 2 - a]pyrimidine, Caryophyllene, 4-Cyclohexene-1, 2-dicarboximide, N-butyl-, cis-, Chola-5,22-dien-3-ol, (3β,22Z)-, Androst-5-en-3-ol, 4,4-dimethyl-, (3β)-, 4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine, Kauren-18-ol, acetate, (4β)-, N-Cyano-N',N',N'',N''-tetramethyl-N-methoxymethyl-1,3,5-triazine-, and Cyclotrisiloxane, hexamethyl-.

ψ,ψ-Carotene, 7,7',8,8',11,11',12,12',15,15'-decahydro- had the highest molecular weight of 546 g/mol., followed by Chola-5,22-dien-3-ol, (3β,22Z)- with 342 g/mol. Phytol had the highest peak area of 10.68%followed by Benzofuran, 2,3-dihydro- with peak area of 8.48%and then 3,6-Dimethyl-5-oxo-1,2,3,5-tetrahydroimidazo 1,2-a] pyrimidine withpeak area of 5.45%. Cyclotrisiloxane, hexamethyl, N-Cyano-N',N',N'',N''-tetramethyl-N-methoxymethyl-1,3,5-triazine and Kauren-18-ol, acetate, (4β)- had the highest retention time of 16.835 seconds, 16.43 seconds , and 16.047 seconds. Some of these compounds have industrial applications when isolated and synthesized artificially. 4-Piperidinone, 1-methyl-for instance is widely used in artificial fibre industry, and can be polymerizable to nylon precursors.2-Ispropyl-1-methoxy-4-methylbenzene is used to enhance food safety and quality. 1,2-Benzenedicarboxylic acid, dicyclohexyl ester are used to produce diester compound known as Phthalates, which is industrially applied in production of plastics. Some such as propanoic acid, 2-(aminooxy) and phytol have well noted physiological activities as bioactive constituents. Propanoic acid, 2-(aminooxy) has antimicrobial, antiviral, antioxidant and ant-inflammatory properties. Phytol has been identified as a precursor for the manufacturing of synthetic forms of vitamin E and vitamin K1. It is also an important component of chlorophyll and a precursor of phtanic acid. De Moraes et al. [64], reported phytol, as a diterpene alcohol from chlorophyll widely used as a food additive and in medicinal fields and possesses promising antischistosomal properties *in vitro* and in a mouse model of *schistosomiasis mansoni*. Olofsson et al. [65] reported phytol as chlorophyll component with anti-inflammatory and metabolic properties. Indole is applied in perfumery, production of tryptophan and a hormone called inodoleacetic acid, which promotes the development of roots in plant cutting. Quinoline, 3-Methylquinoline is

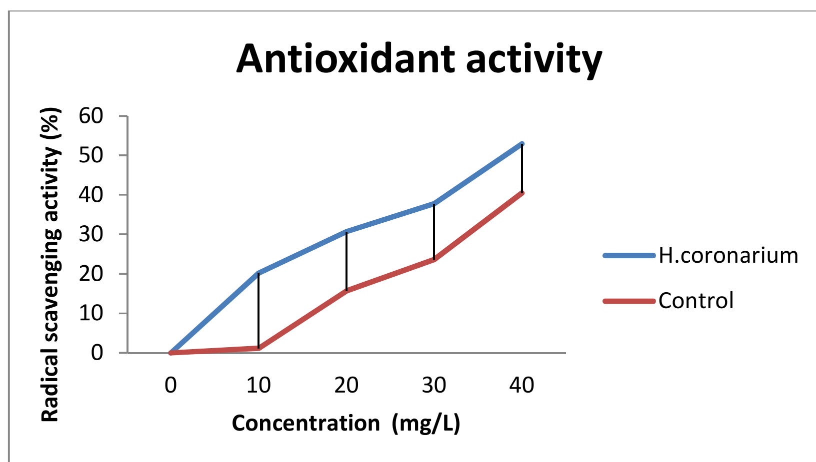

Fig. 1. Antioxidant activity of *H.coronarium* leaf sample

quinoline derivative employed for the synthesis of dyes, as coloring agents to food, pH indicator and other industrial purposes. Indene is carbocyclic analogue of indole, and a microbial metabolite.7-Oxabicyclo[4.1.0]heptan-3-ol,6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl-,7 Oxabicyclo [4.1.0] heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl-, and 5,9-Undecadien-2-one, 6,10-dimethyl-, (E)- have been associated with tobacco and tobacco smoke. The protective roles of oleic acid against cardiovascular insulin resistance and in the early and late cellular atherosclerotic process are noted [66]. Octadecanoic acid, methyl ester plays a role as a metabolite. Piperidine plays a role in the intestine. Caryophyllene is an essential oil in plant. Androst-5-en-3-ol, 4,4-dimethyl-, (3β)- is a structural analogue of androstenediol, and may perform similar function as androstenediol. All the compounds revealed by GC-MS in this study could play a role or the other as bioactive constituents on further examination.

Antioxidants fight the after effects of free radicals. They either slow down the damage caused by free radicals to the cells or prevent their activity in the body. *H. coronarium* leaf sample tends to have better antioxidant activity against that of ascorbic acid used as control in the present study. Both the bioactive phytochemicals and compounds revealed by GC-MS analysis that exhibit physiological activities could be behind the observed antioxidant activity of the leaf sample.

4. CONCLUSION

Alkaloids, flavonoids, cardiac glycosides, phlobatannins, tannins, saponins, and terpenoids were the phytochemicals observed at different concentrations in the *H. coronarium* leaf sample. Also important compounds such as such phytol, indole, ψ,ψ-Carotene, 7,7',8,8',11,11',12,12',15,15'-decahydro-, propanoic acid, 2-(aminooxy), and etcetera showed significant properties of note in this study. The leaf sample exhibited better radical scavenging activity than ascorbic acid used as control in the present study. These phytochemicals and some of the compounds revealed by GC-MS analysis, which are bioactive and exhibit physiologically activity, could be behind the antioxidant activity of the leaf sample. They could as well be responsible for the pharmacological benefits derived from the plant. This study has revealed the pharmacological compounds and antioxidant activity of *H. coronarium* leaf sample.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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