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(RESEARCH ARTICLE)

Characterization and biological activity of some anti-diabetic plants: *Medicago sativa Gongronema latifolium* and *Pterocarpus santalinoides*

Obijuru N.T. *, Nwaoguikpe R.N, Ene A.C. and Igwe C.U.

Department of Biochemistry, Medical Biochemistry unit, Federal University of Technology, Owerri.

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Abstract

Plants are believed to be an essential source of new chemical substances with potential therapeutic effect. The aim of this study is to test for the GCMS analysis and FT-IR analysis of the extract of *Medicago sativa , Gongronema latifolium* and *Pterocarpus santalinoides.* FTIR spectroscopy of all selected extracts confirms the presence of alkanes, alkynes, carboxylic acids, aldehydes and nitriles functional groups while the major bioactive compounds detected in the extracts were protchaechenic acid, hydroxyl benzoic acid , b-Phenyl caffate and kaempherol.

Keywords: *Medicago sativa; Gongronema latifolium; Pterocarpus santalinoides*; Bioactive compounds; Functional groups.,

1. Introduction

The hunt for natural remedies to treat illnesses has drawn a lot of interest, and the most significant source has been medicinal plants[1]. In light of the global upswing in medication resistance, toxicity, side effects, and rising costs of synthetic products, traditional medicine products have played and continue to play bigger roles in the lives of people all over the world. The synergistic and antagonistic pharmacological interaction in poly-herbal medicines provide therapeutic efficacy with little side effects. leaves and stems are abundant in vitamins, minerals, and proteins and help prevent deficiencies of certain nutrients. These plants have several medical applications, including those for treating fungal infections, restoring digestive health, and promoting breastfeeding in nursing mothers. According to several studies [2,3,4], antioxidants are micronutrients that can stop the oxidative damage brought on by free radicals. There are numerous substances with antioxidant action found in medicinal plants. Practitioners of traditional medicine in South East Nigeria use *Medicago sativa*, *Gongronema latifoliun and Pterocarpus santalinoides*, as a folk remedy for treating diabetes mellitus. Hence we made an attempt to study the validity of these folk remedy by investigating the chemical components and functional groups in the three plants.

2. Methods

2.1. Plant Materials

Fresh leaves of the plants *P. santalinoides, M. sativa, and G. latifolium* were gotten from a farm in Umuekwune Ngor-okpala L.G.A., Imo State. Dr. Mbagwu, a taxonomist from Imo State University, authenticated the leaves. The leaves were deposited into the herbarium and voucher numbers IMSUH 551, 552, and 553 were assigned to them.

* Corresponding author: Obijuru NT.

2.1.1. Preparation of Extract

The fresh leaves of the plantswere shed, dried at room temperature of 30^0C and dried leaves reduced to a coarse powder in a mill (Kenwood BL357). The powder (800g) was extracted with 2 L of 80% ethanol using a soxhlet extractor and concentrated in vacuo using rotary evaporator for solvent evaporation. The weight of the dried extracts were measured, and the percentage extract recovery was calculated. The percentage extract of *P.santalinoides, M.sativa and G.latifolium* were 13.5%, 11.1% and 7% respectively. They were stored in a freezer (≤4.0 ^{0}C) until needed.

$$Percentage\ Extract\ Recovered = \frac{\text{dryweight of extract recovered after extraction}}{\text{Initial dryweight of plant part}} \times 100$$

2.2. Characterization of plant extract active component

Characterization of plant extract active component was carried out using GC-MS analysis by the method of[5].

2.2.1. Preparation of sample

The leaves of the plant were air dried at room temperature for about 2 days. The dried leaves were ground dried and powdered samples were subjected to soxhlet extraction method.

2.2.2. Soxhlet Extraction method

Boiling flasks of 500ml were dried in oven at 105 – 110^{0}C for about 30 minutes. It was transferred into a dessicator and allowed to cool. Sample of 100g was poured into the Soxhlet thimble. The boiling flask was filled with about 300ml of ethanol and allowed to reflux for about 4 hours at a temperature of (60^{0}C). The extract was poured into a volumetric flask and allowed to cool. Then, rotatory evaporator was used to separate the solvent (n-hexane) from the oil.

2.2.3. Extraction of phytochemicals

Extract of 1g was weighed and transferred in a test tube and 25ml of ethanol added. The test tube was allowed to react in a hotplate at 60^{0}C for 90mins. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel. The tube was washed successfully with 20ml of ethanol, 10ml of cold water, 10ml of hot water and 3ml of hexane, which were all transferred to the funnel. The extracts were combined and washed three times with 10ml of 10%v/v ethanol aqueous solution. The solution was dried with anhydrous sodium sulfate and the solvent was evaporated. The sample was solubilized in 1000ul of pyridine of which 200µl was transferred to a vial for analysis.

2.2.4. Quantification by GC-MS

The analysis of phytochemical was performed on a BUCK M910 Gas chromatography equipped with HP-5MS column (30 m in length × 250 µm in diameter × 0.25 µm in thickness of film). Spectroscopic detection by GC–MS involved an electron ionization system which utilized high energy electrons (70 eV). Pure helium gas (99.995%) was used as the carrier gas with flow rate of 1 mL/min. The initial temperature was set at 50 °C with increasing rate of 3 °C/min and holding time of about 10 min. Finally, the temperature was increased to 300 °C at 10 °C/min. One microliter of the prepared 1% of the extracts diluted with acetonitrile was injected in splitless mode. Relative quantity of the chemical compounds present in each of the extracts was expressed as percentage, based on peak area produced in the chromatogram.

2.2.5. Identification of chemical constituents

Bioactive compounds extracted from different extracts were identified based on GC retention time on HP-5MS column and matching of the spectra with computer software data of standards (Replib and Mainlab data of GC–MS systems)[6].

2.3. Fourier Transform Infrared Spectrophotometer (FT-IR)

Buck scientific M530 USA FTIR was used for the analysis. This instrument was equipped with a detector of deuterated triglycine sulphate and beam splitter of potassium bromide. The software of the Gram A1 was used to obtain the spectra and to manipulate them. An approximately of 1.0g of samples, 0.5ml of nujol was added, they were mixed properly and placed on a the salt pellet.. During measurement, FTIR spectra was obtained at frequency regions of 4,000 – 600 cm^{-1} and co-added at 32 scans and at 4 cm^{-1} resolution. FTIR spectra were displayed as transmitter values.

3. Result

Result of characterization of active components of *G. latifolium* shows a rich chemical constituents of methylcytisine, pyrimidine – 4,6, cystsine, 17 – oxosparttaine, 5,6-dehydrolupanine, carbonic acid, luparine, sapogenine, catechin, flavon – 3 – ol, Anthocyanindine, spartein, resveratol, 9,12-octadecadienoic acid, aragyrine, baptifoline, 6-octadececanoic, kaempferol and ethyl oleate (Table 1). Results of characterization of active components of *P.santalinoides* (Table 2) shows rich chemical constituents of 1-penten – 3-ol, decanoic acid, butoxyacetic acid, ether, 6-methylheptyl vinyl, docosyl octyl ether, 3-deoxy-d-mannoic lactone, oxazole, 5-hexyl – 2,4 – dimethyl, oxazole, hexadecanoic acid, 1,2 – benzenedicarboxylic acid, n-hexadecanoic acid, 1- octenyl succinic anhydride, cis-13-octadecenoic acid, 1H – cycloprop (e) azulene, cis –vaccenic acid, oleic acid, 2-propenoic acid, 6-octadecenoic acid, 4-thiazolidinone, glycerine acid (ISP –TFA) and octasiloxane. Table 3 shows the chemical constituents of *M.sativa* to include Phenol, 2,4,6 –cycloheptatrien – 1 one, 2-propanone, heptanoic acid, 3-methylcyclopentane-1,2-dione, 2-pyrrolidinone, phenol,2-methoxy, sec-butylamine, 1H-imidazole, octanoic acid, 4 (1H) – Pyridone, 3-pyridinecarboxylic acid, cyclopentanone, 2-hexenal, 1,4:3,6-dianhydro, methenamine, 1,2-benzenenadiol , 3-methoxy, decanoic acid, phenol, 2,6 – dimethoxy , undecanoic acid, 3,5-dimethoxy – 4 – hydroxytoluene, dodecanoic acid and hexadecanoic acid.

Table 1 Chemical Constituents of *Gongronema latifolium* leaf extract

Peak	Retention Time	Component Name	% Concentration	Activity	Ref
1	9.840	Methylcytisine	1.17		
2	12.828	pyrimidine – 4,6	0.88		
3	12.893	Cystsine	0.71		
4	13.113	17 – oxosparttaine	2.79		
5	15.408	5,6-dehydrolupanine	2.01		
6	16.880	carbonic acid	0.76		
7	16.952	Luparine	1.54		
8	17.410	Sapogenine	2.44		
9	17.538	Catechin	7.32	alleviates oxidative stress	7
10	18.743	flavon – 3 – ol	6.73	increases insulin release	8
11	18.980	Anthocyanindine	0.68		
12	19.301	Spartein	24.92	increases insulin release	9
13	19.497	Resveratrol	7.83	elevates the insulin secretion	10
14	19.753	9,12-octadecadienoic acid	2.98		
15	19.869	Aragyrine	4.66	nimproves peripheral and hepatic insulin sensitivity	11
16	19.999	9,12-octadecadienoic acid	2.91		
17	20.137	Baptifoline	5.76		
18	20.900	6-octadececanoic	10.94		
19	30.931	Kaempferol	12.97	promotes insulin secretion	12
20	31.429	ethyl oleate	0.72		

Table 2 Chemical Constituents of *Pterocarpus santalinoides* leaf extract

Peak	Retention Time	Component Name	% Concentration	Activity	Ref
1	11.877	1-penten – 3-ol	19.87	Antioxidant and fragrance agent	
2	12.460	decanoic acid	6.45	Lowers cholesterol level, promotes HDL	[13]
3	12.699	butoxyacetic acid	1.92		
4	14.435	ether, 6-methylheptyl vinyl	7.30	Antioxidant	
5	14.527	docosyl octyl ether	1.60		
6	14.703	3-deoxy-d-mannoic lactone	2.59		
7	15.474	oxazole, 5-hexyl – 2,4 – dimethyl	2.69		
8	16.982	hexadecanoic acid	2.84		
9	17.486	1,2 – benzenedicarboxylic acid	1.37		
10	17.621	n-hexadecanoic acid	12.02	Antioxidant and anti-inflammatory agent	
11	18.208	1- octenyl succinic anhydride	0.76		
12	18.800	cis-13-octadecenoic acid	5.31		
13	19.102	1H – cycloprop (e) azulene	6.08	Antioxidant	
14	19.425	cis –vaccenic acid	12.60	Improves insulin sensitivity and decrease body fat	
15	19.604	oleic acid	5.69		
16	19.915	oleic acid	1.66		
17	20.268	7- pentadecyne	2.33		
18	20.564	2-propenoic acid	0.75		
19	23.602	6-octadecenoic acid	2.60		
20	23.931	4-thiazolidinone	1.54		
21	26.110	glycerine acid (ISP –TFA)	1.24		
22	29.714	Octasiloxane	0.95		

Table 3 Chemical Constituents of *Medicago sativa* leaf extract

Peak	Retention Time	Component Name	% Concentration	Activity	Ref
1	5.216	Phenol	2.29		
2	5.304	2,4,6 –cycloheptatrien – 1 one	1.87		
3	5.414	2-propanone	3.44		
4	5.529	heptanoic acid	0.27		
5	5.718	3-methylcyclopentane-1,2-dione	1.62		
6	6.358	2-pyrrolidinone	0.60		
7	6.550	phenol,2-methoxy	2.28		
8	6.638	sec-butylamine	4.89	Antibiotics	
9	6.755	1H-imidazole	0.47		
10	6.936	octanoic acid	1.39		
11	7.136	4 (1H) – Pyridone	0.99		
12	7.209	3-pyridinecarboxylic acid	1.57		
13	7.894	Cyclopentanone	0.78		
14	7.977	2-hexenal	0.72		
15	8.250	1,4:3,6-dianhydro	1.45		
16	8.277	1,4:3,6- dianhydro	4.299		
17	8.451	Methenamine	22.72	Anti-infective urinary tract	
18	8.961	1,2-benzenenadiol , 3-methoxy	3.89		
19	9.545	decanoic acid	1.77		
20	9.959	phenol, 2,6 – dimethoxy	5.65		
21	10.732	undecanoic acid	1.43		
22	11.076	3,5-dimethoxy – 4 – hydroxytoluene	2.32		
23	11.857	dodecanoic acid	9.82	Lowers risk of heart disease	
24	15.790	hexadecanoic acid	14.99	Anti-inflammatory, Antioxidant, Inhibits lipid peroxidation	[23]
25	16.152	n-hexadecanoic acid	7.89		

3.1. Functional groups of the plants

Results of FTIR spectra of *G.latifolium* sample is recorded in table 4 . From the table of results, the peak value around 797.5683$cm^{-1}$was assigned to C-CI stretching vibration of chloro compound. The absorbance around 1044.199cm^{-1} and 1284.125cm^{-1} were due to C-0 stretching vibration of ether compounds respectively. The peak around 1412.922cm^{-1} was assigned to C=C stretching vibration of ethene compound. The medium band around 1627.421cm^{-1} was assigned to N-H stretching vibration of 1^0 amine compound. The wavelength around 2011.876cm^{-1} and 2198.781cm^{-1} were assigned to C00 stretching vibration of carboxylic acid compound respectively. The peak around 2457.768$cm^{-1}$and 2563.836cm^{-1}corresponds to C-N stretching vibration of nitrile compound respectively. The weak band around 2674.170cm^{-1} and 2850.781cm^{-1} were assigned to C-H stretching vibration of methylene compound. The broad band

around 3062.719cm^{-1}, 3393.036cm^{-1} and 3812.565cm^{-1}were assigned to 0-H stretching vibration of 1^0, 2^0& 3^0 alcoholic compounds respectively.

Table 5 shows the result for *P.santaiinoides* sample, the peak value around 755.7627$cm^{-1}$was assigned to C-CI stretching vibration of chloro compound. The absorbance around 1161.138$cm^{-1}$was assigned to C-0 stretching vibration of ether compound. The adsorption band around 1690.529cm^{-1} was assigned to N-H stretching vibration of 1^0 amine compound whereas the spectra height around 1875.572cm^{-1} was assigned to C-0 stretching vibration cyclic ester compound. The absorption band around 2032.003cm^{-1} and 2109.049cm^{-1} were assigned to C00 stretching vibration of carboxylic acid compound respectively. The peak around 2258.558cm^{-1} was assigned to C-0 stretching vibration of carbonyl compound. The peak value around 2461.492cm^{-1} and 2593.730cm^{-1}corresponds to C-N stretching vibration of nitrile compound respectively. The weak band around 2791.497$cm^{-1}$was assigned to C-H stretching vibration of methylene compound. The strong band around 3020.363cm^{-1}, 3236.081cm^{-1} , 3361.486cm^{-1}, 3538.358cm^{-1}, 3690.035cm^{-1} and 3814.708cm^{-1}corresponds to 0-H stretching vibration of 1^0, 2^0& 3^0 alcoholic compounds respectively.

From the table of results for *M.sativa* sample (Table 6), the peak value around 825.7247cm^{-1} was assigned to C-CI stretching vibration of chloro compound. The absorbance around 1143.633$cm^{-1}$was assigned to C-0 stretching vibration of ether compound. The peak around 1305.183cm^{-1} and 1412.511cm^{-1} were assigned to C=C stretching vibration of ethene compound respectively. The adsorption band around 1615.427cm^{-1} was assigned to to N-H stretching vibration of 1^0 amine compound whereas the spectra height around 1858.289cm^{-1} was assigned to C-0 stretching vibration cyclic ester compound. The absorption band around 2061.864cm^{-1} was assigned to C00 stretching vibration of carboxylic acid compound. The peak around2268.355cm^{-1} was assigned to C-0 stretching vibration of carbonyl compound. The peak value around 2449.902cm^{-1} corresponds to C-N stretching vibration of nitrile compound. The weak band around 2600.965$cm^{-1}$and 2811.642cm^{-1}were assigned to C-H stretching vibration of methylene compound respectively. The peak around 2977.171cm^{-1} was assigned to SCN stretching vibration of thiocyanate compound. The strong band around 3275.852cm^{-1} and 3395.574cm^{-1} corresponds to 0-H stretching vibration of 1^0, 2^0 alcoholic compounds respectively.

Table 4 Functional groups content of *Gongronema latifolium* leaf extract

S/N	Wavelength (cm^{-1})	Functional group	Compounds
1	797.5683	C-CI	Chloro Cl symmetric stretch
2	1044.199	R-O-R	Ether CO symmetric stretch
3	1284.125	R-O-R	Ether CO symmetric stretch
4	1412.922	H_2C=CH	Ethene C=C anti-symmetric stretch
5	1627.421	RNH_3	1^0 amine NH stretch
6	1913.469	R-S- C≡N	Thiocyanate SCN anti-symmetric stretch
7	2011.876	RCOOH	Carboxylic acid C-O stretch
8	2198.781	RCOOH	Carboxylic acid C-O stretch
9	2457.768	R-C≡N	Nitriles C≡N anti-symmetric stretch
10	2563.836	R-C≡N	Nitriles C≡N anti-symmetric stretch
11	2674.170	CH_2	Methylene C-H stretch
12	2850.781	CH_2	Methylene C-H stretch
13	3062.719	RCHOH	1^0 alcohol O-H stretch
14	3393.036	R_2CHOH	2^0 alcohol O-H stretch
15	3812.565	R_3CHOH	3^0 alcohol O-H stretch

Table 5 Functional groups content of *Pterocarpus santalinoides* leaf extract

S/N	Wavelength (cm^{-1})	Functional group	Compounds
1	755.7627	C-CI	Chloro Cl symmetric stretch
2	1161.138	R-O-R	Ether CO symmetric stretch
3	1372.706	H_2C=CH	Ethene C=C anti-symmetric stretch
4	1690.529	RNH_3	1^0 amine N-H stretch
5	1875.572	R-COO	Cyclic Ester C-0 stretch
6	2032.003	RCOOH	Carboxylic acid C-O stretch
7	2109.049	RC00H	Carboxylic acid C-O stretch
8	2258.558	R-C=O	Carbonyl compound COO stretch
9	2461.492	R-C≡N	Nitriles C≡N anti-symmetric stretch
10	2593.730	R-C≡N	Nitriles C≡N anti-symmetric stretch
11	2791.497	CH_2	Methylene C-H stretch
12	3020.363	RCHOH	1^0 alcohol O-H stretch
13	3236.081	RCHOH	1^0 alcohol O-H stretch
14	3361.486	R_2CHOH	2^0 alcohol O-H stretch
15	3538.358	R_3CHOH	3^0 alcohol O-H stretch
16	3690.035	R_3CHOH	3^0 alcohol O-H stretch
17	3814.708	R_3CHOH	3^0 alcohol O-H stretch

Table 6 Functional groups content of *Medicago sativa* leaf extract

S/N	Wavelength (cm^{-1})	Functional group	Compounds
1	825.7247	C-CI	Chloro Cl symmetric stretch
2	1143.633	R-O-R	Ether CO symmetric stretch
3	1305.183	H_2C=CH	Ethene C=C anti-symmetric stretch
4	1412.511	H_2C=CH	Ethene C=C anti-symmetric stretch
5	1615.427	RNH_3	1^0 amine N-H stretch
6	1858.289	R-COO	Cyclic Ester C-O stretch
7	2061.864	RCOOH	Carboxylic acid C-O stretch
8	2268.355	R-C=O	Carbonyl compound COO stretch
9	2449.902	R-C≡N	Nitriles C≡N anti-symmetric stretch
10	2600.965	CH_2	Methylene C-H stretch
11	2811.642	CH_2	Methylene C-H stretch
12	2977.171	R-S-C≡N	Thiocyanate SCN stretch
13	3275.852	RCHOH	1^0 alcohol O-H stretch
14	3395.574	R_2CHOH	2^0 alcohol O-H stretch
15	3482.301	R_2NH	2^0 amine N-H stretch

4. Discussion

4.1. Characterization of the plant active chemical components and determination of the functional groups.

The rich chemical composition of catechin, flavon-3-ol, sparteine, resveratrol, 9,12-octadecadienoic acid, aragyrine, baptifoline, 6-octadececanoic, and kaempferol was revealed by the characterization of the active components of *Medicago sativa, Gongronema latifolium, and Pterocarpus santalinoides* (Table 1,2,3). Tea's primary functional ingredient, catechins, has various health advantages, including the reduction of sugar in diabetes. By enhancing insulin resistance, reducing oxidative stress, regulating mitochondrial function, reducing endoplasmic reticulum stress, providing anti-inflammatory effects, lowering blood sugar, and regulating intestinal function, catechins can alleviate hyperglycemia [14,15,16]. According to studies, catechins reduce fasting blood glucose levels in diabetic rats and inhibit carbohydrate-digesting enzymes including -amylase and -glucosidase [17]. By regulating gut microbiota, modifying inflammatory response, suppressing oxidative stress, and boosting immune function, flavon-3- ol reduces the severity of diabetes complications like retinopathy, nephropathy, neuropathy, vascular dysfunction, cardiomyopathy, coronary diseases, and renal failure. By preserving pancreatic beta cells, increasing insulin signaling, encouraging the pancreas to release insulin, blocking glucogenolysis, inhibiting gluconeogenesis, inhibiting digesting enzymes, and inhibiting carbohydrate metabolizing enzymes, flavon-3-ol lowers blood sugar levels [8]. In type 2 diabetics, L-arginine increased glucose levels, insulin-induced hepatic glucose synthesis, and insulin sensitivity [11]. The study discovered the existence of decanoic acid, a saturated fatty acid that regulates the activity of PPAR receptors, a subfamily of nuclear receptors that is essential for the metabolism of lipids and glucose. Treatments with decanoic acid and its triglyceride form enhance lipid profiles and glucose sensitivity in diabetic animals without causing weight gain [13]. Significantly less miRNA-375 is expressed when resveratrol is present. MiRNA-375 may help treat diabetes mellitus by preventing the body's beta cells from dying through apoptosis, which would maintain enough insulin output in the body [10]. Through PI3K/AKT signal pathways, resveratrol ameliorates uric acid-induced pancreatic beta-cell damage and increases insulin secretion [16]. Sparteine decreases the K+ permeability of the B-cell membrane to promote insulin release [9]. Sparteine caused an electrical activity similar to that of glucose, depolarized the B-cell membrane, and prompted the release of insulin. Similar to an insulin secretagogue, kaempferol encourages insulin secretion. In a study employing the insulin secretagogue glibenclamide as the control substance, it was discovered that kaempferol raised plasma insulin levels and lowered blood glucose in streptozotocin-induced diabetic rats [12]. Mitochondrial Ca^{2+} is crucial for the release of insulin and the metabolism of glucose. The primary mechanism for Ca^{2+} uptake in mitochondria is the mitochondrial calcium monoporter (MCU).

Perhaps the most effective tool for determining the kinds of chemical bonds/functional groups that are present in the phytochemicals is the Fourier Transform Infrared Spectrophotometer (FT-IR). As observed in the annotated spectrum, the wavelength of light absorbed is a key aspect of the chemical bond. It is possible to identify the chemical bonds in a substance by reading its infrared absorption spectra [19]. The current FT-IR results are in agreement with earlier studies done on other plants and confirmed the presence of alkanes, alkenes, amines, carboxylic acids, and alcohols in the three plants [20,21,22,23]. Numerous studies have shown that the FT-IR spectrum is a useful tool for identifying, categorizing, and distinguishing between groups of closely related plants and other species.

5. Conclusion

Alcohol, alkanes, and ester molecules were identified as functional groups of OH, C-H, and C0O in this plant specimen. The chemicals discovered during GC-MS analysis of these plants extract support the traditional practitioner's use of them in the treatment of diabetes mellitus. However, since the detected compounds were previously reported to have significant bio-activity, isolating of each individual component and testing it for biological activity will undoubtedly produce positive results. As a result, using this plant as a source of phytopharmaceutical value is advised.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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