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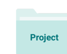

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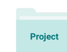

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THAUMATOCOCCUS DANIELLII LEAVES: ITS CHEMICAL COMPOSITIONS, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES.

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ABSTRACT

The preliminary phytochemical investigation of *n*-hexane, ethyl acetate and methanol extracts of *Thaumatococcus daniellii* leaves revealed the presence of fats and oils, terpenoids, flavonoids, steroids and glycosides. The antimicrobial tests against some strains of bacteria and fungi showed inhibitions at moderate to high concentrations. Methanol extract of the plant exhibited low 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity with IC_{50} of 615.14 μg/ml. Gas chromatography-mass spectrometry (GC-MS) characterization of *n*-hexane, ethylacetate and methanol extracts of *T. daniellii* leaves identified ten, thirteen and fifteen compounds, with tetracontane (28.76%) and L-ascorbic acid (15.07%); hexadecanoic acid (21.62%) and γ-sitosterol (11.06%); and naphthalene-1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-,[1R(1.alpha.,7.beta.,8a.alpha.] (26.90%) and hexadecanoic acid (12.60%) being the major compounds respectively. The GC-MS analysis revealed various peaks of bioactive compounds of which the antioxidant and antimicrobial activities of the plant have been attributed to the prominent compounds in synergy with all the other compounds present in smaller quantities in the extracts.

Keywords: *Thaumatococcus daniellii,* Antimicrobial activity, Antioxidant activity, GC-MS analysis, L-ascorbic acid, γ-sitosterol

INTRODUCTION

Thaumatoccocus danielli (Benn.) Benth which belongs to the family of Marantaceae, is a plant species from West Africa known for being a natural source of thaumatin, an intensely sweet protein which is of interest in the development of sweeteners. It is a forest under-storey herb in the Marantaceae native to equatorial Africa, where its leaves are locally used to wrap food and as material for roofing and woven mats (Abbiw, 1990). It grows throughout the hot, humid, tropical rain forest and coastal zone of West Africa. Its natural habitat is the twigs of forest trees. *Thaumatococcus danielli* grows three to four meters in height, and has large, papery leaves up to 46 centimeters long. It bears pale purple flowers and a soft fruit containing a few shiny black seeds. The sturdy leaf petioles are used as tools, building materials and as wrapper for food (Chinedu, 2014). The leaves and seeds have a number of traditional uses. The fruit of the plant is used as a laxative and the seeds used as an emetic and for treating pulmonary problems. The leave sap is used in traditional medicine as antidote against venoms, stings, and bites. Leave and root sap are used as sedative and for treating psychiatric problems (Bentham *et al.,* 1883). Large quantities of the fruits are collected by local people to sweeten over fermented palm wine and sour foods (Franke and Thieme, 1985). The plant is significant such that the leaves have locally been used for wrapping and boiling foods in Ghana and Nigeria (Yeboah, 2002; Yeboah *et al.,* 2003).

The banning of the artificial sweetener, sodium cyclamate in the USA in 1969 has provoked extensive analytical and agronomic research on thaumatin and *T. daniellii* (Adansi, 1970). This research included glass house experiments in the UK (Summerfield *et al.,* 1977; Most *et al.,* 1978) and field plantations in Ghana, Liberia, Nigeria, and Malaysia (Onwueme *et al.*, 1979; Witty and Higginbotham, 1994). There has been surprisingly little research on the ecology and distribution of the species or the local knowledge of people who

currently utilize it (Wojciech *et al.,* 2005), while little pharmacological and phytochemical properties of the plant have been reported. The antibacterial, antioxidant and insecticidal activities of essential oil of the plant have been reported (Adeola *et al.,* 2015; Adeyemi *et al.,* 2014; Anthony *et al.,* 2013). Hence, we report the chemical composition, antimicrobial and antioxidant properties of non-volatile extracts of *T. denielli* leaves.

MATERIALS AND METHODS

Sample preparation: The plant was collected from Oyo town, Oyo state, Nigeria and was identified and authenticated by a plant taxonomist, Mr. Bolu of the Department of Plant biology, University of Ilorin with a voucher number of (UILH/006/1237). The leaves of the plant were sun dried, weighed and extracted using serial exhaustive solvent extraction with three solvents, namely; n-hexane, ethyl acetate and methanol which are of dissimilar polarities.

Phytochemical screening: The screening was carried out using the modified method of Prashant *et al.,* 2011

Antimicrobial assay: Cultures of 10 human pathogenic bacteria and fungi made up of both gram negative and gram positive were used for the assay. The bacteria species used include; *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas Aeruginosa, Salmonellae typhi, Klesiella pneumonia* and the fungi species are; *Candida albicans, Aspergillus niger, Penicillium notatum* and *Rhizopus stolon.* The screening was carried out at the Department of Microbiology, University of Ibadan. Nutrient agar, sabouraud dextrose agar, nutrient broth and tryptone soya agar were used as the media in which the assays were prepared. n-Hexane, ethyl acetate and methanol were used in dissolving the extracts. The antimicrobial standard reference drugs used in this study are Gentamycin (10μg/ml) and Tioconazole (0.7 mg/ml).

Determination of antimicrobial activity

Agar diffusion pour-plate method (Bacteria): An overnight culture of each organism was prepared appropriately from its stock and inoculated each into the sterile nutrient broth of 5 ml, each incubated for 18-24 hrs at 37 °C. From overnight culture, 0.1 ml of each organism was taken and put into 9.9 ml of sterile distilled water to get (1:100) 10^{-2}M inoculum concentration of the organism. From the diluted organism (10^{-2} M), 0.2 ml was taken into the prepared sterile nutrient agar, cooled to about 40-45 ºC, poured into sterile petri-dishes and allowed to solidify for about 45-60 minutes. Using a sterile cork-borer of 8 mm diameter, wells were made according to the number of the test tubes for the experiment. For this work, eight wells were made. The graded concentrations of the extracts were put into the wells accordingly including the controls. The studies were done in duplicates to ascertain the results obtained. The plates were left on the bench for about 2 hrs to allow the extract diffuse properly into the nutrient agar i.e. pre-diffusion. The plates were afterwards incubated for 18-24 hrs at 37 ºC.

Agar diffusion-surface plate method (Fungi): A sterile sabouraud dextrose agar was prepared accordingly and aseptically poured into the sterile plates in duplicates, then left to solidify properly. 0.2 ml of (1:100) 10^{-2} M inoculum concentration of the organism was spread on the surface of the agar using a sterile Petri-dish lid to cover all the surface of the agar. Eight wells were bored using a sterile cork-borer of 8 mm diameter. The graded concentrations of the extracts were put into the wells accordingly including the controls. All the plates were left on the bench for 2 hrs to allow the extract diffuse properly into the agar i.e.pre-diffusion. The plates were incubated at 25 ºC for 72 hrs (Collins *et al.,* 1970).

Antioxidant activity: The ability of the samples to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals was assessed by a standard method (Tekao *et al.*, 1994)**,** adopted with suitable modifications (Kumarasamy *et al.,* 2007). The stock solution of extracts were prepared in methanol to achieve the concentration of 1mg/ml. Dilutions were made to obtain concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90 and 1.99 μg/ml. DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity. The absorbance was measured in duplicate at varying concentrations and the mean absorbance was determined. Parallel

to examination of the antioxidant activity of plant extracts, the value for the standard/control compound (ascorbic acid) was obtained and compared to the values of the antioxidant activity and the percentage inhibitions of the serial concentrations of the methanol DPPH extracts. The percentage inhibitions at different concentrations were determined using the formula (Sies, 1997).

$$\%\ inhibition = \left(\frac{Aof\,control - Aof\,sample}{Aof\,control}\right) \times 100$$

The IC_{50} values were estimated from the plot of % inhibition against concentration, using a non-linear regression algorithm.

Gas chromatography-Mass spectrometry(GC-MS) analysis of the extract: GC-MS analysis of the three plant extracts was performed with Agilent 19091GC plus automatic sampler system coupled with a quadruple Mass Spectrometer 433HP-5MS. Compounds were separated in HP5MS column fused with phenyl methyl silox (length; 30m x 250µm; film thickness 0.25µm). Samples were injected at a temperature of about 250 °C with a split ratio of 10:1 with a flow rate of helium 1ml/min.

RESULTS AND DISCUSSION

The preliminary phytochemical screening of the crude extracts of *Thaumatococcus daniellii* revealed the presence of fats and oils, terpenoids, steroids, tannins, flavonoids, glycosides, saponins as shown in table 1 below. The presence of these metabolites is an indication of the pharmacological activity of the plant.

The antimicrobial results of *n*-hexane extract showed antimicrobial activity on all the test organisms (bacteria and fungi) at different concentrations with relatively high zones of inhibition compared to ethyl acetate and the methanol extracts. Ethyl acetate extract shows no zone of inhibition against two fungi, *Aspergillus niger* and *Penicillium notatum* and is active against other organisms. On the other hand, methanol extract of the plant revealed no antibacterial activity against *Klebsiellae pneumonae* and *Salmonella typhi* and antifungal activity against *Rhizopus stolonifera* (Table 2). Furthermore, the minimum inhibitory results recorded the highest inhibition for *n*-hexane extract at the concentration range of 25-200 mg/ml compared to the inhibition results of ethyl acetate and methanol extracts. These inhibitions indicate that the leaves of *T. daniellii* exhibited antibacterial and antifungal activities and hence can be used for the treatment of various infections caused by these species of bacteria and fungi.

Table 1: Phytochemical screening of hexane, ethyl acetate and methanol extracts of *Thaumatococcus daniellii* leaves

Phytochemicals	Hexane extract	Ethyl acetate extract	Methanol extract
Saponins	-	-	+
Terpenoids	+	+	+
Steroids	+	+	+
Flavonoids	-	+	+
Alkaloids	-	-	-
Fats and oils	+	+	+
Glycosides	-	+	+
Phenols	-	-	-
Carbohydrates	-	-	-
Protein	-	-	-
Anthraquinones	-	-	-
Tannins	-	-	+

Key: + = present; - = absent

Antioxidant activity: The antioxidant activity indicates the presence of certain compounds with structural features that are able to trap and retain free radicals so as to convert them to less toxic compounds in the plant extracts. The extracts' ability (n-hexane, ethyl acetate and methanol) to scavenge DPPH radicals and reducing their effects was analyzed (Table 3 and Figure 1).

The antioxidant activity of *T. daniellii* leaves showed that the percentage inhibition of *n*-hexane extract ranges from high to medium values at high concentrations. For ethyl acetate, there is high inhibition at high concentrations, but medium inhibition of DPPH radicals at low concentrations. The inhibition of methanol has an anomalous value (20 %) at the highest concentration after which it increases to medium inhibition and there is no significant change afterwards. Furthermore, by comparison of the IC_{50} of the three extracts (hexane, ethyl acetate and methanol) with the IC_{50} of the control (ascorbic acid), it was revealed that only methanol extract showed antioxidant activity with IC_{50} value of 615.14 mg/ml. Therefore, only the extract of methanol can effectively reduce the concentration of DPPH radical to 50%.

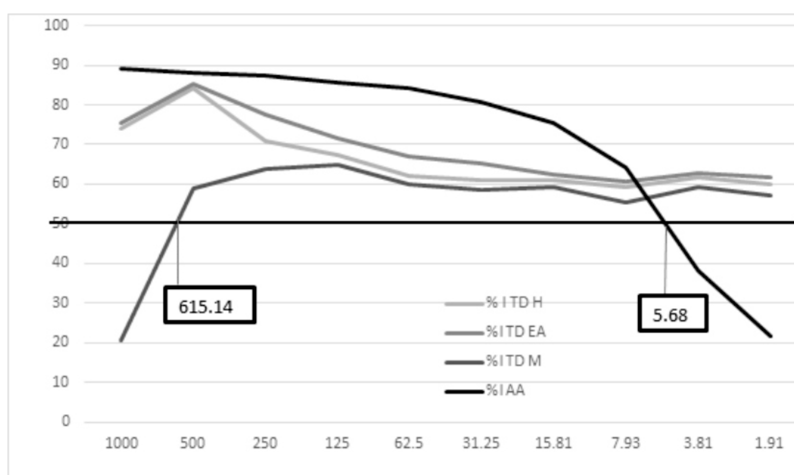
Table 2: Antimicrobial activity of *n*-hexane, ethyl acetate and methanol extracts of *Thaumatococcus daniellii* leaves

Conc of the extract (mg/ml)	S.a	E.c	B.sub	Ps.a	Sal	K.p	C.a	A.n	Pe.n	Rhiz
Hexane extract										
	Mean zone of Inhibition (mm)									
200	23	27	25	27	27	26	20	20	22	21
100	22	23	20	23	23	23	18	18	20	19
50	18	20	18	19	20	20	16	16	18	16
25	16	18	16	16	17	17	14	14	16	14
12.5	14	15	13	13	14	15	12	12	14	12
6.25	10	11	10	10	10	11	10	10	10	10
-ve	-	-	-	-	-	-	-	-	-	-
+ve	37	39	37	37	39	38	27	26	28	27
Ethyl acetate extract										
200	21	21	20	21	20	19	17	-	-	17
100	18	18	18	18	18	16	14	-	-	14
50	16	16	15	15	15	13	12	-	-	12
25	14	14	13	13	13	10	10	-	-	10
12.5	12	12	10	10	10	-	-	-	-	-
6.25	10	10	-	-	-	-	-	-	-	-
-ve	-	-	-	-	-	-	-	-	-	-
+ve	36	38	36	36	40	38	26	28	28	28
Methanol extract										
200	19	19	17	19	_	_	19	17	14	_
100	17	15	14	16	_	_	16	14	12	_
50	14	13	12	14	_	_	14	12	10	_
25	12	10	10	12	_	_	12	10	_	_
12.5	10	_	_	10	_	_	13	_	_	_
-ve	_	_	_	_	_	_	_	_	_	_
+ve	39	38	38	39	39	39	28	27	27	28

Key: S.a = *Staphylococcus aureus;* E.c = *Escherichia coli*; B.sub = *Bacillus subtilis;* Ps.a = *Pseudomonas aeruginosa;* Sal = *Salmonella typhi;* K.p = *Klebsiellae pneumonae;* C.a = *Candida albicans;* A.n = *Aspergillus niger;* Pe.n = *Penicillium notatum;* Rhiz = *Rhizopus stolonifera;* +ve = Gentamycin 10 μg/ml (bacteria); Tioconazole 70% (fungi); -ve = Solvent of dilution

Table 3: DPPH Antioxidant activity and % inhibition of *n*-hexane, ethyl acetate and methanol extracts of *Thaumatococcus daniellii* leaves

Conc. (µg/ml)	A1	A2	A3	MEAN	% I H
1000	0.28	0.302	0.292	0.291±0.0040	74.01
500	0.179	0.175	0.175	0.176±0.0040	84.27
250	0.328	0.326	0.323	0.326±0.0030	70.94
125	0.368	0.366	0.367	0.367±0.0010	67.26
62.5	0.427	0.426	0.426	0.426±0.0020	61.97
31.25	0.439	0.440	0.437	0.439±0.0010	60.87
15.81	0.440	0.437	0.437	0.438±0.0010	60.93
7.93	0.452	0.459	0.457	0.456±0.0010	59.32
3.81	0.431	0.430	0.429	0.430±0.0010	61.64
1.95	0.448	0.447	0.451	0.449±0.0010	59.98
Ethyl acetate extract. Absorbance of control = 0.330					
1000	0.280	0.274	0.270	0.275±0.0010	20.72
500	0.166	0.166	0.167	0.116±0.0020	58.77
250	0.251	0.251	0.252	0.251±0.0010	63.89
125	0.320	0.321	0.320	0.320±0.0020	64.80
62.5	0.368	0.370	0.372	0.370±0.0010	59.94
31.25	0.387	0.390	0.390	0.389±0.0010	58.42
15.81	0.425	0.421	0.420	0.422±0.0010	59.18
7.93	0.438	0.440	0.442	0.440±0.0010	55.37
3.81	0.415	0.418	0.419	0.417±0.0010	59.18
1.95	0.430	0.430	0.433	0.431±0.0010	57.17
Methanol extract. Absorbance of control = 1.265					
1000	0.378	0.382	0.384	0.381±0.0010	20.72
500	0.203	0.194	0.198	0.198±0.0030	58.77
250	0.178	0.173	0.170	0.173±0.0010	63.89
125	0.170	0.169	0.169	0.169±0.0000	64.80
62.5	0.195	0.191	0.192	0.192±0.0020	59.94
31.25	0.198	0.201	0.201	0.200±0.0010	58.42
15.62	0.197	0.196	0.196	0.196±0.0020	59.18
7.8	0.211	0.214	0.219	0.215±0.0010	55.37
3.9	0.201	0.194	0.194	0.196±0.0020	59.18
1.95	0.207	0.203	0.208	0.206±0.0000	57.17



KEYS:
% ITD H: Percentage Inhibition of Hexane extract,
% ITD EA: Percentage Inhibition of Ethyl acetate extract,
%I TD M: Percentage Inhibition of Methanol extract
%I AA: Percentage Inhibition of Ascorbic Acid (control)

Figure 1: IC_{50} Antioxidant activity of *n*-hexane, ethyl acetate and methanol extracts of *Thaumatococcus daniellii* leaves

GC-MS characterization: This analysis characterizes and determines the number of constituents present in the extracts and their relative abundance with retention time for each sample. Each extract components were eluted at different retention time from the gas chromatograph and the mass spectrometer captured, ionized, accelerated, deflected and detected each constituent separately (Gohlke and McLafferty, 1993).

The GC-MS characterization of *n*-hexane extract of *T. daniellii* leaves showed a total of eleven chemical constituents (Table 4). The abundant compounds from the extract are 2-methyloctacosane (% abundance = 15.99), L-ascorbic acid (% abundance = 15.07) and tetracontane (% abundance = 28.76). Hexadecanoic acid (% abundance = 21.62), 9-octadecenamide (% abundance = 17.41), γ-sitosterol (% abundance = 11.06) and urs-2-ene (% abundance = 6.66) are the major constituents from the thirteen chemical compounds of ethyl acetate extract of the plant (Table 5). Methanol extract of *T. daniellii* leaves revealed the principal constituent as: 5-(7a-Isopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-pent-2-en-1-ol, hexadecanoic acid, β-sitosterol and naphthalene,1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1methylethenyl)-{1R (1.alpha.,7.beta.,8a.alpha.)} with their percentage abundance of 22.12, 12.60, 4.91 and 26.90% respectively (Table 6).

Table 4: GC-MS Analysis of the hexane Extract of *Thaumatococcus daniellii* leaves

S/N	Compound	Molecular Formula	Retention time	% Abundance	Molecular weight
1	3,7 –dimethylnonane	$C_{11}H_{24}$	8.760	1.04	256
2	Pthalic acid -2-chloropropylethylester	$C_{13}H_{15}ClO_4$	11.22	1.05	270
3	6,10,14-trimethyl-2-pentadecanone	$C_{18}H_{36}O$	15.259	2.53	268
4	Stearic acid	$C_{18}H_{36}O_2$	16.699	2.17	284
5	Phytol	$C_{20}H_{40}O$	18.299	5.99	296
6	2,6,10,14,18-pentamethyl-2,6,10,14,18-eicosapentaene	$C_{25}H_{42}$	24.182	6.59	342
7	2-methyloctacosane	$C_{29}H_{60}$	24.525	15.99	408
8	L-ascorbic acid	$C_{24}H_{42}O_7$	25.375	15.07	442
9	Chloroacetic acid hexadecyl ester	$C_{18}H_{35}O_2$	25.758	10.72	318
10	Tetracontane	$C_{44}H_{90}$	26.583	28.76	561

Table 5: GC–MS analysis of ethyl acetate extract of *Thaumatococcus daniellii* leaves.

S /N	Compound	Molecular Formula	Retention Time	% abundance	Molecular weight
1.	Dodecanoic acid	$C_{12}H_{24}O_2$	10.896	2.56	200
2	6,10,14-trimethyl-2-pentadecanone	$C_{18}H_{36}O$	15.29	2.99	268
3	n-hexadecanoic acid	$C_{16}H_{32}O_2$	16.779	21.62	256
4	Phytol	$C_{20}H_{40}$	18.300	2.44	296
5	7-hexadecenal (Z)	$C_{16}H_{30}O$	18.566	5.96	238
6	Hexadecanmide	$C_{16}H_{33}NO$	18.897	4.27	255
7	9-octadecenammide	$C_{18}H_{35}NO$	20.536	17.41	281
8	Bis (2-ethylhexyl)phthalate	$C_{24}H_{38}O_4$	22.057	1.78	390
9	γ - sitos terol	$C_{29}H_{50}O$	22.716	11.06	414
10	Urs-2-ene	$C_{30}H_{50}$	23.374	6.66	410
11	Squalene	$C_{30}H_{50}$	24.178	4.66	410
12	Pentatriacontane	$C_{35}H_{72}$	24.725	5.96	492
13	Tetracontane	$C_{40}H_{82}$	26.660	5.80	562

Table 6: GC-MS Analysis of the methanol Extract of *Thaumatococcus daniellii* leaves

S/N	Compound	Molecular Formula	Retention time	% Abundance	Molecular weight
1	6,10,14-trimethyl-2-pentadecanone	$C_{18}H_{36}O$	15.260	2.82	268
2	methyl hexadecanoate	$C_{17}H_{34}O_2$	2.35	2.35	270
3	n-hexadecanoic acid	$C_{16}H_{32}O_2$	12.60	12.60	256
4	Longifolene	$C_{15}H_{24}$	17.884	1.35	204
5	Phytol	$C_{20}H_{40}O$	18.298	2.31	296
6	Octadecanoic acid	$C_{18}H_{36}O_2$	18.792	1.21	284
7	5-(7a-Isopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-pent-2-en-1-ol	$C_{20}H_{34}O$	22.065	22.12	290
8	5-(7a-Isopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-pent-2-enal	$C_{20}H_{32}O$	23.085	5.22	288
9	Naphthalene, 1,2,3,5,6,7,8,8a - octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R - (1.alpha.,7.beta.,8a.alpha.)]-	$C_{15}H_{24}$	23.203	26.90	204
10	β - sitosterol	$C_{29}H_{50}O$	23.508	4.91	414
11	Squalene	$C_{30}H_{50}$	24,171	4.76	410
12	17-(1,5-Dimethylhexyl)-2-hydroxy-10,13-dimethylhexadecahydrocyclopenta[a]phenanthren-3-one	$C_{27}H_{46}O_2$	24.555	5.21	402
13	Acetic acid, 3 -hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-2-yl ester	$C_{17}H_{26}O_3$	24.858	5.36	278
14	n-Octadecyl chloride	$C_{18}H_{37}Cl$	26.642	1.60	288

CONCLUSION

The leaves of *T. daniellii* have been investigated in this research and found to contain interesting metabolites like fats and oils, flavonoids, saponins, steroids, terpenoids and glycosides. The findings of this work showed that the leaves of *T. daniellii* exhibited antimicrobial activity which supports the previous work of Noda *et al.*, 2000. The observed antimicrobial effect may be attributed to the presence of flavonoids as it has been reported that flavonoid has anti-allergic, anti-inflammatory, anti-cancer and anti-microbial effect. This supports the ethno-medicinal uses of *T. daniellii* as antidote against venoms, stings and bites. The GC-MS analyses reveals various peaks of bioactive compounds of which the activity of the plant against bacteria and fungi have been attributed to the prominent compounds in synergy with all the other compounds present in smaller quantities in the extracts.

Conflict of interest.

The authors declare no conflict of interest.

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