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Antidiabetic effects of partitioned methanol extract of *Boswellia dalzielii* (Frankincense tree) on rats

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ABSTRACT

Medicinal plants have been the major source of bioactive phytochemicals employed for the treatment and management of disease since time immemorial. The present study was aimed at investigating the anti-diabetic potentials of various partitioned portions of crude methanol extract of *Boswellia dalzielii* in alloxan-induced diabetic rats. Fresh leaf of *Boswellia dalzielii* was air-dried, pulverized and extracted using cold maceration method with 85% methanol and concentrated to dryness. The crude methanol extract was partitioned using n-hexane, chloroform, ethylacetate and n-butanol to afford portions encoded BMENH, BMECM, BMEEA, BMENB respectively, and were screened for phytochemicals. The portions were evaluated for their anti-diabetic effects on alloxan-induced rats. The phytochemical studies of the crude methanol leaf, stem and root bark extracts revealed the presence alkaloids, cardiac glycoside, flavonoids, saponins, tannins and terpenoids. The partitioned crude methanol leaf extract yielded 14.12 % ($^{w}/_{w}$) *n*-hexane, 6.85 % ($^{w}/_{w}$) chloroform, 4.18% ($^{w}/_{w}$) ethyl acetate and 36.40 % ($^{w}/_{w}$) *n*-butanol extracts respectively. Antidiabetic activity of fractions BMEH, BMEC, BMEE at doses of 200 and 400 mg/kg bd wt. produced significant ($p<0.05$) % inhibitions of glycaemia of 13.51, 18.91, 53.36 and 71.21 respectively, as the highest inhibitions at 400 mg/kg bwt. compared to 52.67 % of Glibenclamide 2.0 mg. (a standard drug). Thus, the leaf of *Boswellia dalzielii* possesses potent antidiabetic activity which increases as the extract is purified. The anti-diabetic effect of the plant in rats may likely be due to the presence of alkaloids, flavonoids, tannins and terpenoids.

Keywords: Diabetes, anti-diabetes, phytochemicals, alloxan, *Boswellia dalzielii*.

INTRODUCTION

Nature has been the source of medicine for thousands of years in the maintenance of human health since ancient time [1]. WHO supports the use of effective and safe remedies and accepts traditional medicine as a valuable resource for primary health care [2]. In addition, majority of the populations in developing countries still have limited access or no access, especially those in remote areas, to modern medicines. Instead traditional medicines were employed for a range of illness including diabetic complications [3]. As a result of renewed interest from western countries in herbal medicines and the increasingly urgent need to develop new effective drugs, traditionally used medicinal plants have recently received the attention of the pharmaceutical and scientific communities [4].

The management of diabetes is a global problem and successful treatment has not yet discovered[2]. More than 50 % of all the drugs currently in use are of natural product origin [5]. Higher plants have been the source of medical agents since earliest time and continue to play a dominant role in the healthcare industry [6]. The physiological effect of medicinal plants lies within some of the chemical substances produced by the plants during secondary metabolism. These are called phytochemical. These secondary metabolites are the compounds in responsible plants for their bioactive properties based on literatures [6].

Diabetes mellitus is a group of metabolic disorder characterized by hyperglycaemia resulting from a defect in insulin secretion, its action, or both. It is basically of two types, namely: Type I, which is an insulin dependent diabetes that affects only 5 % of the diabetic population, while a Type II, which is non-insulin dependent, usually develops in adults over the age of 40 [7] and covers about 95% of the remaining of the diabetic population. Currently, the available treatment for diabetes includes the use of insulin and various oral hypoglycaemic drugs such as sulfonylureas, metformin, glucosidase inhibitors, troglitazone, etc. These drugs have however been reported to produce serious side effects such as liver problems, lactic acidosis and diarrhoea [8]. Diabetes is currently the fifth leading cause of death and has affected around 422 million people [7] and the number of those affected is increasing daily. The World Health Organization

(WHO) predicted that, by 2030 the population affected by the metabolic disorder will be 366 million worldwide [7]. World Ethnobotanical information reported that about 800 plant species have been reported to have antidiabetic potentials. Several plant species have been used for treatment, prevention and management of diabetes by the Natives of Americans, Chinese, South Americans and Asian Indians [8].

Boswellia dalzielii Hutch is a savannah tree which belongs to the family Burseraceae [9] and it is mostly called the "Frankincense tree" [10]. The plant is locally called Hano or Arrarabi in Hausa language (meaning to prevent bad luck), is a popular plant in the Northern part of Nigeria due to its ethno medicinal value. The decocted root bark is used traditionally by the Hausa-Fulanis in Sokoto, Nigeria to treat diabetes [11]. A bark-decoction is used as an antiseptic wash for sores in Ivory-Coast and is an ingredient of a complicated prescription for leprosy [13]. In northern part of Nigeria, the stem bark is boiled and taken for the treatment of fever, rheumatism etc., and the fluid is taken internally for gastrointestinal troubles [13,14]. The Fulanis of northern Nigeria uses cold infusion of the stem bark for management of snake bite [13]. The fresh bark of the root is eaten in Adamawa State, Nigeria, to relieve symptoms of giddiness and palpitations as well as an antidote of arrow-poison [13,14] amongst other numerous medicinal uses.

The conventional drugs used for the treatment of diabetes act by improving insulin sensitivity, increasing insulin production and decreasing the amount of glucose in blood of the patient [15]. The hypoglycaemic effect of pharmacologically active component of plants decrease the effect on α-amylase and has various direct and indirect effects on different blood parameters responsible for development of diabetes[15]. A large number of antidiabetic medicines are available in the pharmaceutical market for the management of diabetes and its related complications; however, currently no effective therapy is available to cure this metabolic disease.

However, due to appalling scientific reports of adverse effects of the drugs used for the treatment and management of diabetes, the efficacies of these compounds are questionable and the demand for new compounds/drugs for the treatment of diabetes would not be overemphasized [16, 17]. Recently, the scientific world has seen a tremendous growth growing interest in the herbal medicine in treatment and management of diabetes both in developing and developed countries, due to their originality from nature and less or no effects [18-20].

In spite of the wide use of various parts of *Boswellia dalzielii* in traditional medicine by the local people in Nigeria for the treatment of diabetes, there is no scientific report on bioassay isolation of active ingredient(s) from the whole plant parts responsible for the antidiabetic efficacy given its rich phytochemical composition [21]. More so, as a consequence of negative scientific reports on the adverse effects of most conventional drugs used in the treatment of diabetes as well as drug resistant incidences, this has necessitated the search for safer and effective drug(s) in *Boswellia dalzielii* for the management of this disease.

MATERIALS AND METHODS

Sample Collection, Identification and Preparation

Fresh leaves stem and root barks of *Boswellia dalzielii* were collected from Gulantabar, Song Local Government Area, Adamawa State, Nigeria in the month of January, 2019. The plant materials were transported in a wool sacked-bag to the University of Maiduguri, identified and authenticated by Prof. S.S. Sanusi, a Plant Taxonomist in the Department of Biological Science, University of Maiduguri, Borno State, Nigeria to be of *Boswellia dalzielii,* given a voucher specimen of #341and deposited at the herbarium of the Postgraduate Research Laboratory of Chemistry Department, University of Maiduguri, Maiduguri, Borno state. The fresh plant samples were cleaned and air-dried under shade at room temperature for ten (10) days and were rendered free of foreign material through manual picking. The air-dried plant materials were pulverized using a mortar and pestle and then subjected to these analyses below.

Plant Extraction

The powdered plant material (2 kg) each of the leaf was macerated using 7.5 litres of 85 % methanol for 72 hours with periodic shaking and allowing to stand at room temperature for a proper dissolution of soluble plant chemicals. The liquid mixture of the extract was filtered using a clean muslin followed by filtration using 200mm diameter of Watmann No. 1 filter paper. The crude extracts were concentrated to dryness by the use of rotary evaporator at 40^{o}C. The crude methanol extract was weighed, coded BMLE - *Boswellia dalzielii* methanol leaf extract.

Partitioning of Methanol Extract of *B. dalzielii*

Three hundred grammes (300g) of the crude leaf methanol extract was further partitioned exhaustively using n-hexane, chloroform and ethyl acetate sequentially. The residue was suspended in distilled water and then partitioned with n-butanol. The fractions obtained were evaporated to dryness at reduced pressure using a rotary evaporator and then coded BMENH, BMECM, BMEEA, BMENB, – as n-hexane, dichloromethane, ethyl acetate and n-butanol respectively. The percentage (%) yield, colour, texture and weight of each partitioned portion were noted, labelled and preserved in a dessicator until required for further studies.

Preliminary Phytochemical Screening

The partitioned fractions of the methanolic leaf extract of *Boswellia dalzielii* were screened qualitatively for phytochemical constituents using standard procedures [22]. These include Alkaloids, anthraquinones, cardiac glycosides, flavonoids, tannins, terpenes and saponins.

Pharmacological Investigations of the Partitioned Portions of Crude Methanol Leaf Extract of *Boswellia dalzielii*

Experimental Animals

All the experiments performed on laboratory animals in this study followed standard procedure for the treatment of animals. The animals were handled according to the International Guiding Principle for Biomedical Research involving animals [23].

A total of one hundred and sixty-four (164) albino rats weighing (100-180) g of both sexes were obtained from the Animal House of the Faculty of Pharmacy, University of Maiduguri, Borno State. The study was conducted at the Department of Pharmacology, Faculty of Veterinary Medicine, University of Maiduguri, Maiduguri, Borno State. The animals were fed with standard feed and allowed water, at their will.

Ethical Approval

All experiments were conducted in accordance with the National Institute of Health Guidelines for the Care and use of Laboratory Animals (NIH Publications No.80-23) as revised in 1996.

Extract Fractions and Alloxan Preparation

The partitioned portions of crude methanol extract and alloxan (2 g) each were dissolved in 10 ml distilled water to give a stock solution of 200 mg/ml.

$$\text{Stock solution} = \frac{2000\ \text{mg}}{10\ \text{ml}} = 200\ \text{mg/ml}$$

$$\text{Volume to be administered} = \frac{\text{Dose x Body Weight in Kg}}{\text{Concentration of the Extract in mg}}$$

Test for Hypoglycaemic Activity

The animals were fasted for 12 hr but were allowed access to water before and throughout the duration of the experiment. The blood of each rat was withdrawn from the tip of the tail of each rat under mild anesthesia and the fasting blood glucose (FBG) was estimated with a blood glucometer (Accu-Check, Roche, Germany) at the end of their fasting period at a time taken of zero (0 hr).

Evaluation of Extracts Activity in Alloxan-induced hyperglycaemic Rats

Method described by Uzor *et al.* [24] and Ezeigbo [25] were adopted in this study, with little modification. The animals were fasted for 12 hrs with water *ad libitum* and injected intraperitoneally with freshly prepared alloxan monohydrate (150 mg/kg) in ice-cold 0.9% saline (NaCl) solution. They were given 5 ml of 10% dextrose solution to overcome the drug induced hypoglycaemia and were provided with standard laboratory diet *ad libitum* after one hour. The FBG was checked before and 72 hrs after alloxan injection by withdrawing blood from the tip of the tail of each rat under mild anesthesia. The FBG was measured as described above. The animals were considered diabetic when the FBG is raised beyond 200 mg/dl. They were segregated into five (5) groups of four animals in each. Group-I served as the normal i.e, animals fasted and water was given *ad libitum*. Group II served as the negative control and received vehicle (normal saline, 2 ml/kg, p.o.), Group III and IV were administered 250 and 500 mg/kg of portioned portions of n-hexane, chloroform, ethylacetate and n-butanol of crude methanol extract of *B. dalzielii*. Group-VI, glibenclamide (2 mg/kg, p.o.). Blood glucose concentration was measured after 0, 1 hr, 3hrs, 5 hrs and 7 hrs of administration of single dose of each of the treatments.

Statistical Analysis

Results of pharmacological studies were analysed using GraphPad Prism. 2016 Model, Version 7.0 for windows. One-way Analysis of Variance (ANOVA) test followed by Dunnet's Multiple Comparison test was used to analyse and compare the results at a 95% confidence level. Results were expressed as mean ± standard error of mean (SEM).

RESULTS

The Partitions Profile of Crude Methanol Leaf Extract of *B. dalzielii*

The crude methanol leaf extract of *B. dalzielii* which was partitioned with n-hexane, chloroform, ethyl acetate and n-butanol with colours ranging from dark green colour of the n-hexane fraction, to brown of the n-butanol, and were mostly gummy masses except for the n-butanol fraction which was powdery. The n-butanol fraction also had the highest yield (36.40 %), while n-hexane, chloroform and ethyl acetate had 14.12 %, 6.85 %, and 4.18 % respectively. The result of the extraction profile is shown on Table 1:

Table 1: The Partitioning Profile of *B. dalzielii* Methanol Leaf Extract

S/N	Extract	Mass (g)	%Yield ($^{w}/_{w}$)	Colour	Texture
1	*n*-hexane	42.37	14.12	Dark green	gummy
2	Chloroform	20.55	6.85	Dark green	gummy
3	ethyl acetate	12.53	4.18	Greenish grey	gummy
4	*n*-butanol	109.20	36.40	Reddish brown	Powdery

Phytochemical Constituents' Analysis of Partitioned Portions of Methanol Leaf Extract of *B. dalzielii*

The phytochemical screening of the partitioned portions of the crude methanol leaf extract of *B. dalzielii* with n-hexane, chloroform, ethyl acetate and n-butanol revealed the presence of cardiac glycosides, flavonoids, saponins, tannins and terpenoids in the n-butanol fraction while n-hexane had the least phytochemicals present. The result of the phytochemical screening of the gradient extraction is shown in Table 2.

Table 2: Photochemical screening of partitioned portions (chloroform, *n*-hexane, ethyl acetate and *n*-butanol) of methanol crude leaf extract of *B. dalzielii*

S/N	Phytochemical Test	BMEH	BMEC	BMEE	BMEB
1	Test Tor Carbohydrates	-	-	-	+
2	Test for Tannins	-	-	+	+
3	Test for Steroids/Triterpenes	-	+	+	+
4	Test for Flavonoids	-	-	+	+
5	Test for Saponnins	+	+	+	+
6	Test for Steroidal Nucleus/cardinolides	+	+	+	+
7	Test for Terpenoids	+	+	+	+

Key: BMEH – *n*-hexane partitioned portion of methanol leaf extract of *Boswellia dalzielii*
BMEC– chloroform partitioned portion of methanol leaf extract of *Boswellia dalzielii*
BMEE– ethyl acetate partitioned portion of methanol leaf extract of *Boswellia dalzielii*
BMEB – *n*-butanol partitioned portion of methanol leaf extract of *Boswellia dalzielii*

Effect of n-Hexane, Chloroform, Ethylacetate and n-Butanol Partitioned Portions of crude Methanol Extract of *B. dalzielii* on Alloxan-induced Wistar Strain Rats

The wet partition method for fractionation of the methanol leaf extract of *B. dalzielii* afforded BMEH, BMEC, BMEE and BMEB which were tested for antidiabetic activity at two dose levels of 200 mg/kg and 400 mg/kg and the results shown in Table 4.17, Table 4.18, Table 4.19 and Table 4.20.

The fraction encoded BMEH at 200 mg/kg and 400 mg/kg produced a non-significant reduction (14.87 % and 13.51 % rspectively) in FBG after 7 hrs (Table 4.17).

The BMEC produced a dose-dependent significant (P<0.05) reduction in the FBG of the animals with reductions of 15.84 and 18.91 % at 200 mg/kg and 400 mg/kg doses respectively. 52.68 %).

The antidiabetic activity of both BMEH and BMEC are however, lower than that of BMEE at the same doses of 200 mg/kg and 400 mg/kg which had a remarkable significant decrease of 50.25 % and 53.36 %, equaling the effect of the standard drug glibenclamide (52.68 %) at 2.0 mg.

Similar pattern was also observed for the BMEB fraction, but had the most remarkable significant % reduction of FBS levels in alloxan induced diabetes in rats, which produced dose-dependent non-significant (P <0.05) reduction in the blood glucose (66.16 % and 71.21 % for 200 mg/kg and 400 mg/kg respectively) after 9 hrs when compared to the other fractions stated above, the -ve control (diabetic rats) (5.32 %), and standard control drug glibenclamide (52.68 %) used for this study at 2.0 mg concentration.

The results show that the various fractions possess antidiabetic activity, however, the activity resides more with the n-butanol fraction

Table 4.17: Effect of n-hexane partitioned portion of crude methanol extract of *Boswellia dalzielii* on alloxan-induced Wistar strain rats

S/N	Treatment (mg/kg)	Basal FBG (mg/dL)	Fasting Blood Glucose (FBG) concentration (mg /dL) Time (hr) after treatment					% Inhibition of Glycaemia
			0	1	3	5	7	
1	Normal	73.50±5.24	73.00±4.92	75.75±5.02	72.75±4.33	70.50±5.64	78.00±2.74	-
2	D.C (150)	73.50±5.24	263.00±16.50	286.50±25.18	262.00±10.80	253.80±4.33	249.00±9.25	5.32
3	200	75.00±3.24	465.50±38.17	419.00±38.76	413.50±41.65	402.80±42.06	396.30±40.91*	14.87
4	400	72.00±4.08	503.30±4.73	495.50±40.40	486.00±36.22	471.00±41.24	435.30±42.85*	13.51
5	Glibenclamide (2.0mg)	73.25±1.90	307.50±32.69	305.0±32.16	240.30±46.48	215.30±4.33	145.50±28.93*	52.68

Results expressed as Mean ±SEM (n=4). *P<0.05, as compared with control group (One way, ANOVA followed by Dunnet's t-test, 2 sided). % Inhibition of glycaemia denote percentage reduction of blood glucose from 0 h. Basal FBG=FBG before induction of diabetes; D.C = diabetic control

Table 4.18: Effect of chloroform partitioned portion of crude methanol extract of *Boswellia dalzielii* on alloxan-induced Wistar strain rats

S/N	Treatment (mg/kg)	Basal FBG (mg/dL)	Fasting Blood Glucose (FBG) concentration (mg /dL) Time (hr) mean±SEM after treatment					% Inhibition of Glycaemia
			0	1	3	5	7	
1	Normal	73.50±5.24	73.00±04.92	75.75±5.02	72.75±4.33	70.50±5.64	78.00±2.74	-
2	D.C (150)	73.50±5.24	263.00±16.50	286.50±25.18	262.00±10.80	253.80±4.33	249.00±9.25	5.32
3	200	75.00±3.24	260.30±15.97	248.15±14.79	243.00±13.86	232.80±8.23	220.00±8.17*	15.48
4	400	72.00±4.08	460.00±39.66	435.00±44.05	427.30±49.03	432.80±8.23	373.00±45.98*	18.91
5	Glibenclamide (2.0mg)	73.25±1.90	307.50±32.69	305.00±32.16	240.30±46.48	215.30±4.33	145.50±28.93*	52.68

Results expressed as Mean ±SEM (n=4). *P<0.05, as compared with control group (One way, ANOVA followed by Dunnet's t-test, 2 sided). Figures in parenthesis denote percentage reduction of blood glucose from 0 h. Basal FBG=FBG before induction of diabetes; D.C= diabetic control

Table 4.19: Effect of ethylacetate partitioned portion of crude methanol extract of *Boswellia dalzielii* on alloxan-induced Wistar strain rats

S/N	Treatment (mg/kg)	Basal FBG (mg/dL)	Fasting Blood Glucose (FBG) concentration (mg /dL) Time (hr) after treatment					% Inhibition of Glycaemia
			0	1	3	5	7	
1	Normal	73.50±5.24	73.00±4.92	75.75±5.02	72.75±4.33	70.50±5.64	78.00±2.74	-
2	D.C. (150)	73.50±5.24	263.00±16.50	286.50±25.18	262.00±10.80	253.80±4.33	249.00±9.25	5.32
3	200	75.00±3.24	295.50±15.12	260.30±22.89	213.50±11.21	181.30±17.15	147.00±16.29*	50.25
4	400	72.00±4.08	283.00±13.18	261.80±22.67	227.00±13.89	185.50±2.63	132.00±15.69*	53.36
5	Glibenclamide (2.0mg)	73.25±1.90	307.50±32.69	305.00±32.16	240.30±46.48	215.30±4.33	145.50±28.93*	52.68

Results expressed as Mean ±SEM (n=4). *P<0.05, as compared with control group (One way, ANOVA followed by Dunnet's t-test, 2 sided). % Inhibition of glycaemia denote percentage reduction of blood glucose from 0 h. Basal FBG=FBG before induction of diabetes; D.C= diabetic control.

Table 4.20: Effect of n-butanol partitioned portion of crude methanol extract of *Boswellia dalzielii* on alloxan-induced Wistar strain rats

S/N	Treatment (mg/kg)	Basal FBG (mg/dL)	Fasting Blood Glucose (FBG) concentration (mg /dL) Time (hr) after treatment					% Inhibition of Glycaemia
			0	1	3	5	7	
1	Normal	73.50±5.24	73.00±4.92	75.75±5.02	72.75±4.33	70.50±5.64	78.00±2.74	-
2	D.C (150)	73.50±5.24	263.00±16.50	286.50±25.18	262.00±10.80	253.80±4.33	249.00±9.25	5.32
3	200	75.00±3.24	495.00±39.26	358.00±52.49	322.80±48.52	235.00±50.74	167.50±39.02*	66.16
4	400	72.00±4.08	531.50±21.56	368.50±18.77	278.3±44.95	183.50±44.93	153.00±44.75*	71.21
5	Glibencla-mide (2.0mg)	73.25±1.90	307.50±32.69	305.00±32.16	240.30±46.48	215.30±4.33	145.50±28.93*	52.68

Results expressed as Mean ±SEM (n=4). *P<0.05, as compared with control group (One way, ANOVA followed by Dunnet's t-test, 2 sided). % Inhibition of glycaemia denote percentage reduction of blood glucose from 0 h. Basal FBG=FBG before induction of diabetes; D.C=diabetic control.

DISCUSSION

The phytochemical studies of the methanol leaf, stem and root barks of *B. dalzielii* revealed chemical constituents such as alkaloids, cardiac glycosides, flavonoids, saponins, tannins and terpenoids. The presence of these phytochemicals in the plant parts are in agreement with the reports of Mamza *et al*. [21] and Balogun *et al*. [26]. The reported hypoglycemic activity of the plant extract could be associated with these phyto-compounds present. These compounds are also known to exert pharmacological and antagonistic effects [27]. Terpenes have been reported to pose important biological activities, such as analgesic [27], anticonvulsant [28], cardiovascular [29] antimalarial and antibacterial [22]. Alkaloids have pharmacological applications as anesthetics and CNS stimulants [30].

The glucose lowering effect by the partitioned portions of the methanol crude extract in a dose-independent manner may reflect uptake of the bioactive chemicals through saturable active transport [31]. Although, the n-butanol partitioned portion had a more remarkable antidiabetic activity. This could be due to high content of phenolic compounds such as flavonoids, saponins and tannins present in the fraction. These class of compound have been reported to possess excellent antidiabetic properties [32, 33, 34].

The possible mechanisms underlying the hypoglycemic activity exhibited by *B. dalzielii* could be inhibition of intestinal absorption of glucose, facilitation of glucose-induced insulin release, enhancement of peripheral glucose uptake, promotion of the regeneration of β-cell of islets of Langerhans as well as amelioration of oxidative stress [35]. Thus the order of the activity in in increase order of polarity n-hexane<chloroform<ethylacetate<n-butanol.

Diabetes mellitus is one of the rapidly growing endocrine disorders with major complications affecting populations living throughout the world [36]. The pathophysiological mechanisms are being scrutinized and the knowledge on heterogeneity and complexity of this disease is being advanced. Accordingly, the search for more appropriate therapy is also being under way. In line with that, traditional medicines are used substantially by diabetic patients across the globe [37] and medicinal plants have been identified to be a target for scientists to come up with newer and better therapeutic options in the future.

Moreover, during the past few years many phytochemicals responsible for anti-diabetic effects have been isolated from the plants, such as alkaloids, glycosides, flavonoids, saponins polysaccharides, glycolipids, peptidoglycans, amino acids etc. Quite a number of plants has been used traditionally in treatment of diabetes and some have been proven scientifically to have hypoglycemic activity [39, 40, 41, 42] and these compounds are responsible for the antidiabetic activity.

CONCLUSION

In conclusion, the present study revealed that, the n-butanol partitioned portion of crude methanolic leaf extract of *Boswellia dalzielii* was more efficacious than the n-hexane, chloroform, and ethylacetate in respective, while the portions had activity in increasing order of polarity. Therefore, further antidiabetic study on the n-butanol of the leaf extract of *Boswellia dalzielii* should be carried out in order ascertain the actual bioactive constituent(s) responsible for this effect.

Conflict of Interest

The authors declare no conflict of interest.

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