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RESEARCH ARTICLE

PHYTOCHEMICAL STUDY AND ANTI-HYPERGLYCEMIC EFFECTS OF *ALLIUM SATIVUM* BULBS GROWING IN SUDAN

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ABSTRACT

Objective: The present study represents the effect of the extracts of *A. sativum* bulbs growing in Sudan on glucose reuptake by rats hemidiaphgrams, and; to investigate the anti-hyperglycemic effect of the extracts on glucose-loaded Wistar albino rat.

Methods: *A. sativum* bulbs were powdered and extracted continuously by Soxhlet apparatus using 96% ethanol to obtain the total crude ethanolic extract. The extracts with increasing polarity were successively prepared with petroleum ether, chloroform, ethyl acetate, and methanol using the Soxhlet apparatus. The extracts were screened for their phytochemical constituents. Their anti-hyperglycemic effect was evaluated *in vitro* on glucose reuptake; by using isolated rats hemi-diaphgrams after loading the fasting rats with glucose and; *in vivo* by investigate the anti-hyperglycemic effect of the extracts on glucose-loaded Wistar albino rat. Their effects were compared to control rats administered with the vehicle and to a standard group administered with Metformin standard drug.

Results: The result revealed the presence of flavonoids, alkaloids, terpenoids, tannins and saponins. The *in vitro* anti-hyperglycemic results showed that, the glucose reuptake by isolated hemi-diaphragm was found to be 10.01 mg/g regarding the control group. Whereas, the glucose reuptake by hemi-diaphragm treated with Metformin, crude ethanolic extract, petroleum ether extract, chloroform extract, ethyl acetate extract and methanolic extract was found to be 17, 11.39, 24.11, 19.07, 15.66, 12.71 mg/g respectively. The *in vivo* anti-hyperglycemic effect of the petroleum ether extract with the highest *in vitro* activity revealed lowering of blood glucose level on glucose-loaded rat with 25 and 60 mg/dL after two and four hours of loaded respectively when treated with 200mg/kg of the extract. Regarding the high dose of 400mg/kg lowering of blood glucose level was found to be 30 and 53 mg/dL after two and four hours of loaded respectively. Whereas, the lowering in the blood glucose level of the rats treated with Metformin drug was found to be 23 and 48 mg/dL after two and four hours respectively. GC-MS analysis of the petroleum ether extract, with highest anti-hyperglycemic activity showed the presence of Methyl linolate (42.75%), Hexadecanoic acid, methyl ester (10.54%), Methyl α-linolenate (8.36%), Dotriacontane (6.83), Tetrapentacontane (6.33), Methyl 18-methylnonadecanoate (4.8), Phenol,2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl] (3.25), Methyl 20-methyl-heneicosanoate (2.70), Pentatriacontane (2.13) and many other minor compounds. The most of these compounds are well known for their anti-diabetic Activity.

Conclusions: The study conclude the significant anti-hyperglycemic effect of *A. sativum* bulb extracts. In addition to the petroleum ether extract was found to posses anti-hyperglycemic activity more than the other extracts and Metformin standard drug.

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INTRODUCTION

Diabetes mellitus is considered as one of the five leading causes of death in the world (Nabila Helmy Shafik, 2016). In Sudan the actual number of people with diabetes is not known, the incidence of diabetes is progressively increasing. Consequently, diabetes is now one of the major health problems in Sudan resulting in about 10% of all hospital admissions and mortality, this figure is underestimated as patients who died at home or were unable to reach hospital due to lack of transportation or economic constraints were not included. The main problems of diabetes in Sudan include the lack of efficient diabetes care centers and the high cost of anti-diabetic treatments as causes for default from regular care or treatment (Ahmed, 2000; Awad Mohamed Ahmed, 2001; World Health Organization, 2014). Diabetes have been treated with several medicinal plants extracts for a long time, and

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meanwhile improving health conditions without causing serious adverse reactions and reduce associated side effects of the synthetic anti diabetic drugs (http://bioweb.uwlax.edu/bio203/2010/erbes_sara/classification.htm). Therefore, the search for more effective and safer anti-diabetic agents derived from plants has become an interest area of active research, fortunately they show a bright future in the therapy of diabetes mellitus and its complications. *Allium sativum* is an important medicinal plant of Liliaceae family commonly known as garlic, it is one of the oldest plants widely used as a medicine for thousands years. The bulbs are the most frequently used part as spice and in medice, they are generally used as dried or powdered plant in form of tablets and capsules (Sheela, 1992), they are used traditionally as a dietary supplement, for high cholesterol, heart disease, high blood pressure, prevention of certain types of cancer, including stomach and colon cancers. They have been used to treat all manner of illnesses including fevers, diabetes, rheumatism, intestinal worms, colic, flatulence, dysentery, liver disorders, tuberculosis, facial paralysis, high blood pressure and bronchitis (Sheela, 1992; Benkeblia, 2004; Mathew, 1996 and Harenberg, 1998). The bulbs recognized as pain reliever, anti-worm, anti-bacterial, anti-fungal, anti-viral, antioxidant, anti-cancer, lowering of blood pressure, blood glucose and cholesterol, prevent blood clots, spasms and provide a liverprotection. The essential oil of *A. sativum* showed a potent antimicrobial effect, anti hyperlipidemic, antiplatelet activity, antioxidant activity and antineoplastic activity (Ali, 1995; Saravanan, 2004; Thomson, 2003; Gazuwa, 2013; Amadi, 2009; Ameh, 2013; Ameh, 2010 and Mikail, 2010). The present study was conducted to investigate and compare the anti-hyperglycemic effect of the crude ethanolic (96%) extract, and successive extracts of petroleum ether, chloroform, ethyl acetate and methanol of *A.sativum* growing in Sudan.

MATERIALS AND METHODS

Plant Material Collection and Preparation: *A. sativum* bulbs were collected from Omdurman local vegetable market, Sudan in a fresh form. Authentication was performed by taxonomist at the National Centre for Research, Medicinal and Aromatic Plants Research Institute (MAPRI), Khartoum, Sudan. The voucher specimens were deposited at Pharmacognosy Department, Faculty of pharmacy, University of Medical Science and Technology (UMST). The cloves (bulbs) of A. Sativum was peeled, sliced and dried away from sun light for two weeks, then grinded by mortar and pestle.

Phytochemical Screening Test : The phytochemical constituents of the plant material were detected using standard procedure as described by Sofowora, (1993) (Sofowora, 1993), and Farhat *et al*., (2011). The extract was prepared by boiling 20 grams of powdered plant material with 250 ml of 70% ethanol; then filtered and used for phytochemical screening.

Anti-hyperglycemic Activity Test

Preparation of the Extracts: Extraction of *A. sativum* was carried out usig Soxhlet extractor apparatus according to method descried by Sukhdev *et. al.* (2008), (Sukhdey, 2008) by using 96% ethanol for the crude ethanolic extract. Successive extracts were prepared using solvents of increacing polarity; petroleum ether, chloroform, ethyl acetate and methanol succsively. The percentage yield was calculated.

The Effect of *A.sativum* Extracts on Glucose Reuptake using Isolated Rats Hemi-diaphragm Tissues: Glucose uptake by rat hemi-diaphragm was estimated by the methods described by Walaas (1952); Chattopadhyay *et. al.,* (1992) and Hayder *et. al.,* (2015) with minor modification. Fourteen (14) albino rats from the National center for research (Khartoum) were fasted overnight and killed by decapitation. The diaphragms were dissected out quickly before death with minimal trauma and placed in beaker containing physiological solution (tyrode) supplied with oxygen to ensure tissue survival. Each diaphragm was then divided into two halves and one half is placed in a test tube (each test tube has different composition). All test tubes were placed in an incubator, and tissues were incubated for 30min at 37^{o} C ±1 in an atmosphere of approximately 95% oxygen and 5% CO_2 with hand shaking every 3min. Test on each set was repeated 4 times.

Seven sets each containing four numbers of graduated test tubes (n=4), were taken as follows:

Group 1 (Control): 2ml tyrode solution with 2% glucose; Group 2 (Standard): 2ml tyrode solution with 2% glucose + 0.62ml Metformin (0.1%); Group 3 (Crude ethanolic extract): 2ml tyrode solution with 2% glucose + 1.38ml of 96% crude ethanolic extract (0.1%); Group 4 (Petroleum ether extract): 2ml tyrode solution with 2% glucose + 1.38 ml of petroleum ether extract (0.1%); Group 5 (Chloroform extract): 2ml tyrode solution with 2% glucose + 1.38 ml of chloroform extract (0.1%); Group 6 (Ethyl acetate extract): 2ml tyrode solution with 2% glucose + 1.38 ml of ethyl acetate extract (0.1%). Group 7 (Methanol extract): 2ml tyrode solution with 2% glucose + 1.38 ml of methanolic extract (0.1%).

The volumes of all test tubes were made up to 4 ml with distilled water to match the volumes. The hemi-diaphragms were taken out and weighed. Glucose uptake was calculated as the difference between the initial and final concentration of the glucose in the incubation medium.

The Effect of *A. sativum* Bulbs Petroleium Ether Extract on Glucose Loaded Rats

The effect of petroleium ether extract of *A. sativum* bulbs on glucose loaded rats was conducted as described by Hayder *et. al.,* (2015), and Abdolreza *et al*., 2012. Healthy wistar albino rats (72-92g) were housed under environmental conditions at temperature (25 ± 2^{o}C) and light and dark (12/12). They were feed with standard pellet diet. Rats were fasted for 18 hours and divided into four groups each of 4 rats. All rats received glucose at dose of 2g/kg body weight intraperitoneal. Control group (group 1) was administered with distilled water orally at dose of 10ml/kg body weight. The standard group (group 2) has received metformin orally at dose of 88mg/kg body weight. The 3 rd group (low dose) received orally 200mg/kg of *A. sativum* petroleum ether extract, the 4^{th} group (high dose) received orally 400mg/kg of *A. sativum* petroleum extract. Blood samples were taken Retro-orbitally and centrifuged, then the serum glucose level was monitored at 0 hour, 1 hour, 2 hours and 4 hours after treatment of the groups under study.

GC/MS Analysis

The analysis was carried out in Alawiya Imam center in the university of Medical Science and Technology (UMST), in Khartoum. 2ml of petroleumt ether extract of *A.* sativum was

put into test tube, then 7 ml of alcoholic NaoH was added and Shaked by vortex for 3 minutes. The mixture was leaved over night, then the second day, 2ml from supersaturated NaCl + 2ml of normal hexane were added and shaked for three minutes. Finally the hexane layer was collected. 5µL of hexane layer was taken and then diluted with 5ml diethyl ether. 1gram of sodium sulphate was added as drying agent. The solution was filtered through syringe filter 0.45 µm and the filtrate was directly injected to the GC-MS vial and 1µL was directly injected to the GC/MS-QP2010 ultra (SHIMADZU) Japan with Mass spectroscopy detector (MS). The separation was carried out on Rtx-5MS, Lenght (30m), Diameter (0.25mm), and thickness (0.25µl), with helium as carrier gas in the split mode by direct injection method. The temperature of injection port was maintained at 300^{o}C. The pressure of 96.2 Kpa with flow of 1.50 mL/min was maintained with linear velocity of 44.7cm/sec and total flow of 50mL/min. The temperature of the detector was set at 300^{o}C Temperature was maintained at 60^{o}C for five min and then increased at a rate of 3^{o}C to reach the final temperature of 300^{o}C for 2 minutes. Analysis time was 27 minutes. The identification of different components was achieved from their mass spectra and retention time (RT), compared to those in NIST library. The fragmentation pattern of major constituents was carried out and their m/z value was compared with those obtained in the Mass spectra and result was recorded (http://webbook.nist.gov/ chemistry/).

RESULTS AND DISCUSSIONS

The results of phytochemical screening of *A. sativum* bulbs extract are reported in table 1. The result showed the presence of alkaloids, flavanoids, terpeniods, saponnins and tannins which were found to be reported in the plant (Mikail, 2010).

Table 1. Phytochemical screening result of *A.* sativum bulbs

Phytochemical	Results
Alkaloids	+
Flavonoids	+
Terpenoids	+
Saponins	+
Tannins	+
Cardiac glycosides	-
Anthraquinones	-
Bitter principles	-
Steroids	-
Carbohydrates	-
Reducing sugar	-

The plant extracts estimated for their anti-hyperglycemic effect were prepared by continuous extraction method using Soxhlet apparatus. The percentage yields are shown in Table 2.

Table 2. Percentage yield of *A. sativum* bulbs extracts

Extract	Yield percentage (%)
Crude ethanol	16.18
Petroleum ether	0.368
Chloroform	0.196
Ethyl acetate	0.149
Methanol	10.746

The effects of *A. sativum* bulbs extracts on glucose uptake by isolated rat hemi-diaphragms are shown in table 3 and figure 1. The lower effect (11.39 mg/g) was found when used the total crude ethanoic extratct in comparison to all other treated groups.

In succesive extracts, petroleum ether extract has a highest effect (24.11 mg/g) on the glucose uptake even more than the effect of Metformin standard drug (17 mg/g). In addtion to, the chloroform extract seems to have activity (19.07 mg/g) more than Metformin but less than petroleum ether extract. Whereas, the ethyl acetate have activity (15.66 mg/g) less than that of Metformin drug. Methanolic extract show activity (12.71 mg/g) lower than all other succesive extracts but higher than the total crude extract. Based on the polarity of solvents, the observed activity displays a wide variation in correlation with the polarity of extract solvents used, and the activity was found to be proportional to the polarity of the solvents. This may be attributed to the fact that the chemical constituents responsible for the anti-diabetic activity are of high polarity. These results seem to be comply with preliminary phytochemical analysis results obtained (Table 1) which revaled the presence of chemical constituents of high polarity. From the results it appeared that, the total crude ethanolic extract has the activity lower than the all succesive extracts, this is comply with the antagonistic action reported on the constituents of *A. sativum* bulbs (27).

Table 3. The effect of *A. sativum* bulbs extracts on glucose uptake by isolated rat hemi-diaphragms

Inucubation media	Glucose uptake mg/g/30min
Control	10.01
Metformin	17
Crude ethanolic extract	11.39
Petroleum ether extract	24.11
Chloroform extract	19.07
Ethyl acetate extract	15.66
Methanolic extract	12.71

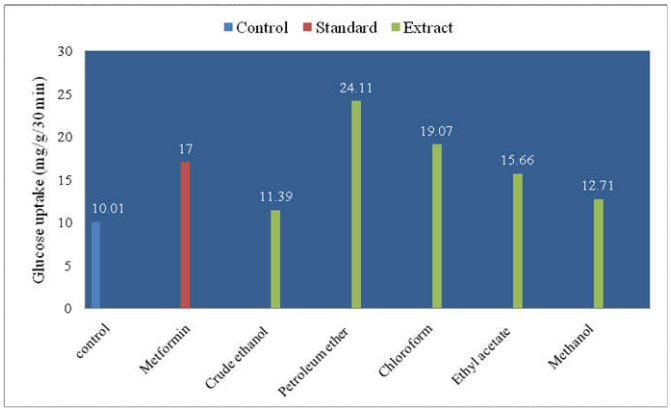

Figure 1. The effect of *A. sativum* bulbs extracts on glucose uptake (mg/g/30 min) by isolated rat hemi-diaphragms

In this study, the petroleum ether extract of *A. sativum* bulb, with the highest activity on the isolated rat hemi-diaphragms (table 3 & fig 1), was evaluated *in vivo* for blood glucose lowering activity on glucose-loaded rats. The obtained result was in table 4 and figure 2. The results revealed lowering of blood glucose level on glucose-loaded rat with 25 and 60 mg/dL after two and four hours of loaded respectively when treated with 200mg/kg of the extract. Regarding the high dose of 400mg/kg lowering of blood glucose level was found to be 30 and 53 mg/dL after two and four hours of loaded respectively. Whereas, the lowering in the blood glucose level of the rats treated with Metformin drug was found to be 23 and 48 mg/dL after two and four hours respectively. From these results it appeared that the petroleum ether extract of *A. sativum* bulb growing in Sudan have activity higher than

Table 4. The effect of *A. sativum* bulbs petroleum ether extract on glucose loaded rats

Group	Serum glucose concentration (mg/dL)			
	0 hour	1 hour	2 hour	4 hour
Control	84.75	154.75	149.75	142.5
Metformin	85.25	131.25	108.75	83.25
Low dose (200mg/kg)	84.75	124.75	99.75	64.75
High dose (400mg/kg)	85.5	112.5	82.75	59.5

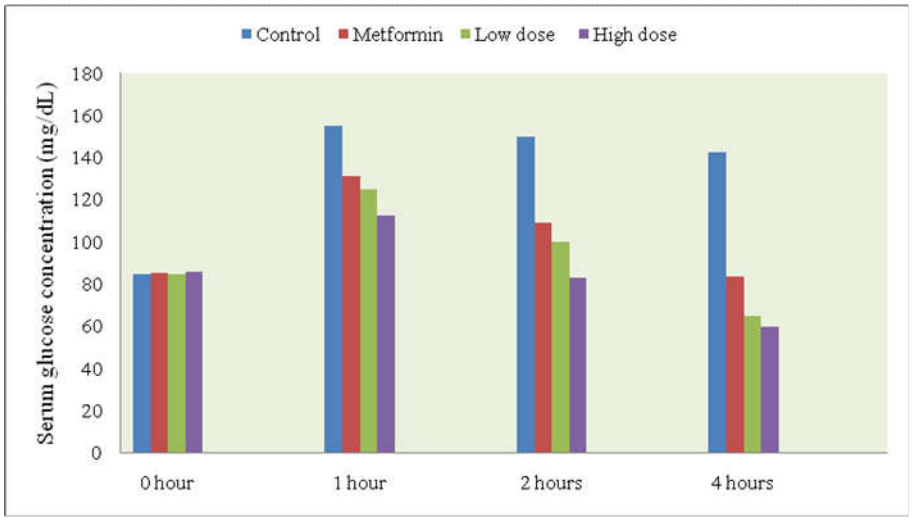

Figure 2. The effect of *A. sativum* bulbs petroleum ether extract Low dose (200mg/kg) and High dose (400mg/kg) on glucose loaded rats

Table 5. The GC-MS Result of petroleum ether extract of *A. sativum* bulbs

Peak No.	R. time	Area %	Compound Name	Formula	M.wt
1	7.157	0.42	Alpha-Terpineol	$C_{10}H_{18}O$	154
2	7.244	0.07	Cyclohexanol,1-methyl-4-(1-methylethylidene)	$C_{10}H_{18}O$	154
3	9.446	0.17	Phenol, 2-methoxy-3-(2-propenyl)	$C_{10}H_{12}O_2$	164
4	11.427	0.16	Dodecanoic acid ,methyl ester	$C_{13}H_{26}O_2$	214
5	13.751	0.32	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242
6	14.563	0.10	6-Octadecenoic acid ,methyl ester	$C_{19}H_{36}O_2$	296
7	14.828	0.20	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	256
8	15.185	0.13	2-propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-,methyl ester	$C_{11}H_{12}O_4$	208
9	15.564	0.24	Eicosane	$C_{20}H_{42}$	282
10	15.622	0.51	Methyl hexadec-9-enoate	$C_{17}H_{32}O_2$	268
11	15.666	0.32	9-hexadecenoic acid, methyl ester,(z)-	$C_{17}H_{32}O_2$	268
12	15.857	10.54	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
13	17.519	42.75	Methyl linolate	$C_{19}H_{34}O_2$	294
14	17.586	8.36	Methyl α-linolenate	$C_{19}H_{32}O_2$	292
15	17.777	1.12	Methyl stearate	$C_{19}H_{38}O_2$	298
16	17.911	1.67	9,12-octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280
17	19.539	4.8	Methyl 18-methylnonadecanoate	$C_{21}H_{42}O_2$	326
18	20.484	3.25	Phenol,2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl]	$C_{23}H_{32}O_2$	340
19	20.897	2.13	Pentatriacontane	$C_{35}H_{72}$	492
20	21.164	2.70	Methyl 20-methyl-heneicosanoate	$C_{23}H_{46}O_2$	354
21	21.975	0.58	Oxirane,heptadecyl-	$C_{19}H_{38}O$	282
22	22.415	6.33	Tetrapentacontane	$C_{54}H_{110}$	758
23	22.898	6.29	Unidentified		
24	23.820	6.83	Dotriacontane	$C_{32}H_{66}$	450
		100			

Metformin drug towards the both doses which was found to be compatable with the in vitro result (table 3 and figure 1) that found in the isolated hemi-diaphragms tissue. Dose of 400 mg/kg extract showed the highest hypoglycemic effect on the induced hyperglycemia in rats even at four hours after the glucose load, which explained that, the effect was found to be dose dependent. In statistical analysis at zero time there is no significant difference between all groups of rats, which is a good base that all rats are relatively similar. After one hour of administration there is significant difference between the control and the other three groups which received standard and extracts, which confirm their hypoglycemic action.

The high dose 400 mg/kg extract show the activity higher than the low dose 200mg/kg which continue even after 4 hours with p-value (0.000). The low dose 200mg/kg extract also show good hypoglycemic effect with decrease p-value over time, 0.0023, 0.002, 0.001 which mean the increase in the strength of its activity which explained that, the effect was found to be time dependent. From the results it appeared that, the anti-hyperglycemic activity of petroleum ether extract of *A. sativum* bulbs was higher than Metformin drug even at low dose and after 4 hours which was found to be compatable with the *in vitro* result (Table 3 and Figure 1) that found in the isolated hemi-diaphragms tissue.

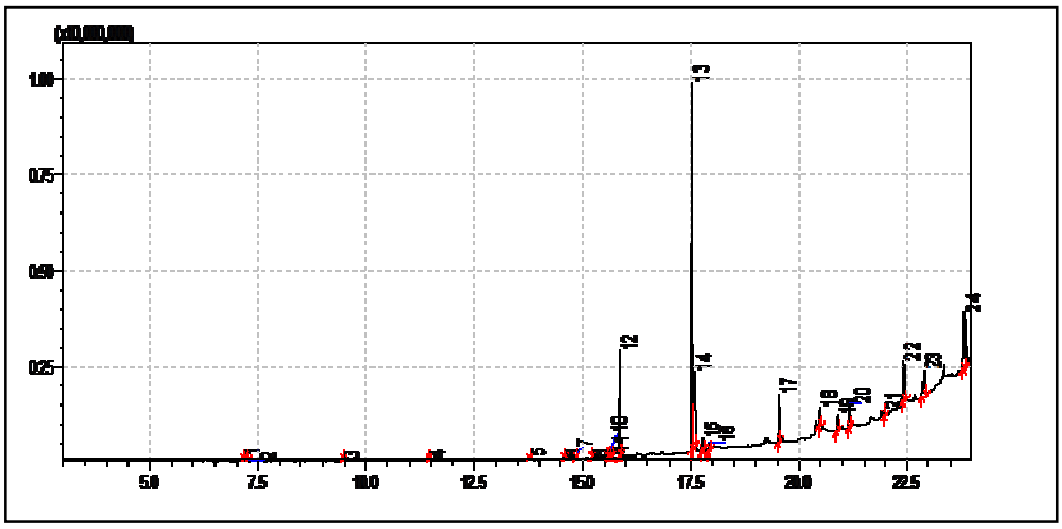

Figure 3. GC-MS chromatogram of petroleum ether extract of *A. sativum* bulbs

The activity was found to be dose dependent and time dependent. The petroleum ether extract, with highest anti-hyperglycemic activity was analyzed chemically using GC-MS analysis. The result was shown in in table 5 and figure 3. GC-MS result revealed the presence of twenty four compounds. The major compounds were found to be Methyl linolate (42.75%), Hexadecanoic acid, methyl ester (10.54%), Methyl α-linolenate (8.36%), Dotriacontane (6.83), Tetrapentacontane (6.33), Methyl 18-methylnonadecanoate (4.8), Phenol,2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl] (3.25), Methyl 20-methyl-heneicosanoate (2.70), Pentatriacontane (2.13) which are well known for their anti-diabetic activity (Chandra Mohan, 2015; Li1, 2014; Mussie Sium, 2017; Zuraini Ahmad, 2012 and Nabawya Ibrahim, 2009).

Conclusion

The study concluded that, the extracts of *A. sativum* bulbs growing in Sudan have significant *in vivo* anti-hyperglycemic effect, and enhanced the glucose re-uptake by the isolated hemi-diaphragms tissue. The extracts could therefore be a potential source for anti-diabetic drug.

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