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RESEARCH ARTICLE

EVALUATION OF THE PHYTOCHEMICAL COMPOSITION, ANTIOXIDANT PROPERTIES, AND *IN VIVO* ANTIHYPERGLYCEMIC EFFECT OF *COCCINIA GRANDIS* LEAF EXTRACT IN MICE

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Abstract

Background: Diabetes mellitus (DM) is a disease described as a high level of blood glucose level and caused by Insulin Deficiency called (Type I). Abnormal metabolism of carbohydrates in connection with insufficient insulin production is called Type II (insulin resistance). As synthetic drugs are generated for the management of different types of DM in injectable and oral dosage forms undesired side effects are being recorded.
Objective: The research side moves nowadays to the safer chemical components with hypoglycemic effects. Using natural remedies, particularly those derived from medicinal plants, to treat diabetes has emerged as a promising alternative treatment.
Methods: This study investigated the phytochemicals constituents, and anti-hyperglycemic and anti-oxidant activities of *Coccinia grandis* leaves extract. Where the leaves of *Coccinia grandis* were collected and the extract containing ethanol was made. The initial phytochemical screening concerning the plant powder was done using standard procedures. Sixteen mice were divided into four groups, group (1) received normal saline, group (2) received standard oral hypoglycemic agent (glimepiride), group (3) received Glucose 50%, and group (4) received ethanolic extract. Extract doses were calculated according to the mice's body weight.
Results: Antioxidant activity for the plant extract was found to be (62 ± 0.03 %) The Anti-hyperglycemic effect was determined by calculating the reduction in glucose levels as a function of time.
Conclusion: The outcomes showed that the pharmacological impact of *C. grandis's* extract reduces the blood glucose levels than the normal metabolic process and when compared to the standard oral hypoglycemic agent.
Keywords: Anti-oxidant, anti-hyperglycemic, *Coccinia grandis*, diabetes mellitus, *in-vivo*.

INTRODUCTION

Plants are one of the most important sources of pharmaceuticals, and 2,50,000 higher plant species that have been identified globally are utilized medicinally as a result of the statistical value of 80,000 species for different clinical manifestations[1]. According to World Health Organization (WHO) research, based on estimates, over 80% of people on the earth are directly dependent on conventional medical treatments. The traditional usage of herbal items involves treating patients' basic medical needs primarily using plant-based remedies[2]. Additionally, it is thought that 40% of the pharmaceutical businesses rely exclusively on medicinal plants therefore always new natural products with high efficacy were requested to maximize the therapeutic outcomes[3,4]. One of the most prevalent chronic illnesses in the world is diabetes mellitus, which can be treated naturally, especially herbal medicines, have emerged as a potential alternative therapy[1]. Herbal medicine is important in the treatment of diabetes mellitus because it contains a lot of secondary metabolites that act with different mechanisms of action to reduce the level of blood

glucose directly or even indirectly[5]. *Coccinia grandis* L. Voigt (Family: *Cucurbitaceae*) has been used for many years as a traditional medicine, especially in India and Sri Lanka[6]. The anti-diabetic properties of this medicinal plant were evident in all of its components, and its putative methods of action include, organizing metabolic enzymes, boosting insulin secretion, restoration of antioxidant enzymes, improvement of the lipid profile, suppression of digesting enzymes, and stimulation of -cell development in the pancreas where all of those mechanisms play an important role in reducing blood glucose (BG) level[7,8]. The hypoglycemic efficacy of *C. grandis* fruit has been studied in a diabetic animal model developed by Alloxan. The ethanol extract shows a decrease in the blood glucose level by inhibiting glycogen phosphorylase, increasing liver glycogen stores, and decreasing glucose absorption from the stomach[9]. It has been demonstrated that the methanolic extract of *C. grandis* and *Salvadora oleoides* leaves together produces a hypoglycemic effect. The hypoglycemic characteristics of the petroleum ether and ethyl acetate extracts of *C. grandis* are attributed to the triterpenes, alkaloids, flavonoids, and carotene present in this plant[10]. Glutathione, superoxide dismutase, catalase, glutathione peroxidase, and glutathione-S-transferase were all decreased by *C. grandis* leaf extract (CLEt), indicating the antioxidant capacity of CLEt[11]. The present study aims to study the phytoconstituents, measure the antioxidant activity, and evaluate the antihyperglycemic effect of *C. grandis* leaf extract.

MATERIALS AND METHODS

Collection and extraction of plant material

C. grandis leaf material was gathered straight from El-jurief farms (Eastern Khartoum - Sudan). Then it was authenticated by the Medicinal and Aromatic Plants and Traditional Medicine Research Institute. Leaves were cleaned and air dried in the shade, then ground to powder using mortar and pestle. Total 29 gm of powdered material was weighed and subjected to hot extraction by Soxhlet for 8 hours using ethanol (96%)[12], the yield percentage was calculated.

Preliminary phytochemical analysis

Phytochemical screening for the detection of alkaloids, saponins, flavonoids, tannins, anthraquinone, cardiac glycosides, sterols/triterpenes, and coumarins were being investigated using the standard recommended screening tests (Mayer's, Wagner's, and Dragendorff's tests), (Froth test), (Sodium hydroxide test), (Ferric chloride test), (Bontrager's test), (Kedde's and Keller-Killani tests), (Liberman and Salkwoski tests) for the detection of those[13,14].

Antioxidant activity

The antioxidant radical scavenging was discovered, using 2.2 Di(4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) to react with the extract for thirty minutes at 37°C. The DPPH concentration was maintained at 300 µM. While DPPH was being produced in ethanol, the test samples were dissolved in DMSO. After incubation, a multi-plate reader spectrophotometer was used to detect absorbance at 517 nm. The percentage of radical scavenging activity was calculated by comparing it to a control group that received DMSO treatment. Every test and analysis was carried out three times[18].

Anti-hyperglycemic activity

Sixteen mice were obtained from the animal house and separated into four groups (4 rats in each group) for the running of the bio-assay experiment. The standard was being prepared as the single dose dissolved in normal saline then the dose administrated to the experimental animal according to their body weight[15]. Both standard and tested extracts were given per IV route. The *in-vivo* antihyperglycemic effect of *C. grandis* was evaluated using a method reported by Widdatallah *et al*.[17]. The sixteen overnight fasted rats were loaded with:

Group 1: Normal saline 10 ml/kg (Control (-ve), Group 2: Glucose 2 ml/kg, Group 3 (Standard): Glimepiride 6.4 mg/kg, (Control (+ve), Group 4: Tested extract.

In-vivo study was running under standard laboratory conditions, 12 hours incubated in light and 12 hours in dark. Examined mice subjected to a series of 0.5 ml, 1 ml, and 1.5 ml of dissolved extract in normal saline (0.1 g/1 ml) in the group (4) within a time interval of 15 minutes and equivalent amounts of each tested material for the rest groups as mentioned previously. The mice were kept after the results were recorded and the mortality rate was determined[18,19].

Toxological profile

The ten healthiest mice were examined with a series of 0.5 ml, 1 ml, and 1.5 ml of dissolved extract in normal saline (0.1 g/1 ml), each mouse was injected with an interval 15 min. The mice were kept under standard laboratory conditions with 12-hour light and 12 hrs dark cycles. The mortality rates were observed.

Determination of chemical composition using Gas Chromatography-Mass Spectroscopy technique

The sample's qualitative and quantitative analysis was completed using Gas- Chromatography/Mass-Spectrometer (GC/MS) technique model (GC/MS-QP2010-Ultra) (Shimazu, Japan), capillary column (Rtx-5ms-30 m x 0.25 mm x 0.25 micro meter)[16], and serial number 020525101565SA. The split mode was used to inject the sample, and helium was used as the carrier gas at a flow rate of 1.61 ml/min. A temperature program was initiated, starting at 50ºC and increasing at a rate of 7ºC/min to 180ºC. and a rate of 8ºC/min to 280ºC The injection port temperature was 300°C, the ion source temperature was 200ºC, and the interface temperature was 250°C as the final temperature after a 3-minute hold period. The sample was examined in the 40–500 m/z range using scan mode, and the entire run took 34 minutes[17]. By comparing retention durations and mass fragmentation patents with those kept in the National Institute of Standards and Technology (NIST) library, the components of the sample were identified and then the list of Components was recorded[17].

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS version 26.0) software was used to analyze the data obtained from the questionnaires. Descriptive statistics (mean, median, frequency, and percentages) were used to evaluate the data. ANOVA-test and Pearson Chi-

Square were used to measure the significance. A *p*-value <0.05 was considered statistically significant. The results are presented as N (%) and mean value ± standard deviation.

RESULTS

Phytochemical screening

The plant gave a high percentage yield of 44.4% by the hot extraction method and was found to be a sufficient quantity for the experiments running by it. The qualitative phytochemical screening of *C. grandis* leaves extract chemical compounds detected showed that the plant consists of saponins, coumarins, tannins, flavonoids, cardiac glycosides, cholesterol, and terpenoids in terms of twenty-six chemical compounds, while alkaloids and anthraquinones were not detected.

Antioxidant activity

The antioxidant activity of plant extract was measured using DPPH scavenging activity, the ethanolic extract showed moderate antioxidant activity (62±0.03 %) compared to the Propyl gallate standard antioxidant agent which possessed RSA% of 91±0.01%.

Antihyperglycemic activity

The results showed that *C. grandis* extract has anti-hyperglycemic activity. It was found that the extract can reduce the level of blood glucose at a considerable rate when compared to the normal rate of decline of glucose blood levels (Table 1).

Table 1: Induction of control negative, extract, and Glimepiride drug (control positive).

Sample	Mice	Initial before induction of glucose	After 5 Minutes of induction of glucose	After 45 minutes of induction of glucose
Control –ve	1	106	283	124
	2	107	133	112
	3	135	178	128
	4	124	237	143
Average		**118**	**207.75**	**126.75**
Glimepiride (Control +ve)	1	104	181	55
	2	81	153	51
	3	109	207	89
	4	100	180	94
Average		**98.5**	**180.25**	**72.25**
Extract	1	171	291	280
	2	95	87	25
	3	121	223	25
	4	155	314	61
Average		**135.5**	**228.75**	**97.75**

The administration of ethanolic extract significantly reduced blood glucose levels in hyperglycemic mice compared to the common anti-hyperglycemic drug (Glimepride). According to the literature review, a study conducted by Attanayake *et al*., reported that in diabetic rats treated with plant extract and glibenclamide, there was a statistically significant decrease in the percentage of glycosylated hemoglobin along with a concurrent increase in the levels of blood insulin and C-peptide. (*p*-value less than 0.05)[24]. The findings from the study of Attanayake AP closely correlate with the results of the current research.

The results showed that *C. grandis* L. extract has anti-hyperglycemic activity. It was found that the extract (group 4) can reduce the level of blood glucose at a considerable rate when compared to the of decline of oral hypoglycemic agent (group 3) and normal homeostatic rate of glucose (group 2) within the time interval of 5-45 minutes of the experiment from average of blood glucose level at the beginning of *in-vivo* trial of each group equal to 228.75 mg/dl, 180, 25 mg/dl, 128.74 mg/dl respectively, as represent in Figure 1, and Figure 2.

Kinetic profile

The kinetic profile of the impact of the plant extract of *C. grandis L* following the zero-order kinetic (Linear pharmacokinetics) is also known as dose-independent and concentration-independent where the response was exerted time-dependent and after an hour of the dose administered no further drop in blood sugar levels was being appeared among the mouse group taken a specific dose of the plant extract.

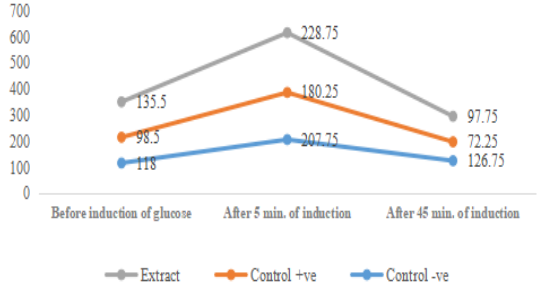

Figure 1: Anti-hyperglycemic effect of plant extract, control +ve and –ve.

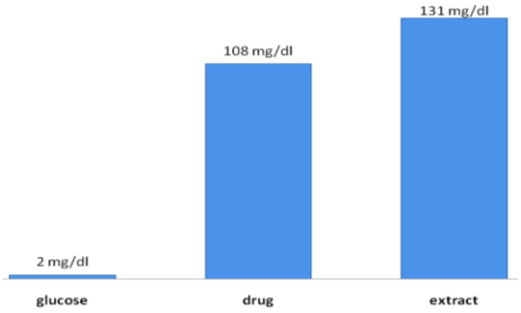

Figure 2: Average Hypoglycemic effect of glucose, drug, and extract groups of mice respectively.

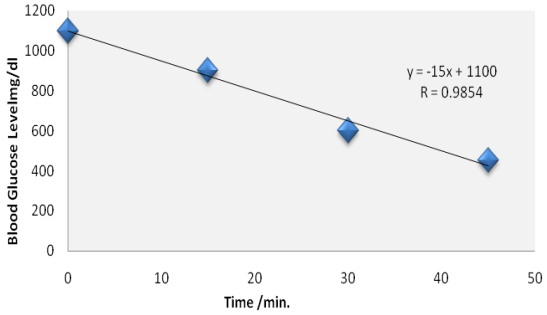

Figure 3: Blood glucose concentration (mg/dl) versus Time (min.) kinetic profile of the plant.

Table 2: Chemical constituents detected in *C. grandis* leaf extract using GC-MS.

S. N.	Name of compound	R.Time	R. Index	Formula	Area %
1.	Phnol,4-ethenyl-,acetate	9.751	1263	$C_{10}H_{10}O_2$	2.96
2.	2-Methoxy-4-vinylphenol	11.319	1293	$C_9H_{10}O_2$	2.61
3.	Oxirane, tetradecyl-	20.386	1707	$C_{16}H_{32}O$	2.70
4.	Clonitazene	20.961	3064	$C_{20}H_{23}CIN_4O_2$	1.78
5.	Pentdecanoic acid, 14-methyl-, methyl ester	21.424	1814	$C_{17}H_{34}O_2$	1.56
6.	n-Hexadecanoic acid	22.078	1968	$C_{16}H_{32}O_2$	15.66
7.	Hexadecanoic acid, ethyl ester	22.308	1978	$C_{18}H_{36}O_2$	0.48
8.	9,12-Octadecanoic acid, Methyl este, (E,E)-	23.456	2093	$C_{19}H_{34}O_2$	1.08
9.	8,11,14-Eicosatrienoic acid, methyl ester (Z,Z,Z)	23.508	2300	$C_{21}H_{36}O_2$	0.35
10.	11-Octadecanoic acid, methyl ester	23.560	2085	$C_{19}H_{36}O_2$	1.32
11.	Phytol	23.786	2045	$C_{20}H_{40}O$	4.07
12.	Cyclopentaneundecanoic acid, methyl ester	23.899	1921	$C_{17}H_{32}O_2$	0.40
13.	9-Octadecynoic acid	24.079	2184	$C_{18}H_{32}O_2$	13.31
14.	9,12,15-Octadecatrienoic acid, (Z,Z,Z)	24.133	2191	$C_{18}H_{30}O_2$	2.86
15.	Oleic acid	24.162	2175	$C_{18}H_{34}O_2$	4.94
16.	Octadecanoic acid	24.419	2167	$C_{18}H_{34}O_2$	3.10
17.	2-Tetradecanone	25.640	1549	$C_{14}H_{28}O$	1.02
18.	Undecanal,2-methyl-	27.392	1338	$C_{12}H_{24}O$	1.07
19.	Glycerol 1-palmitate	27.797	2482	$C_{19}H_{38}O_4$	9.25
20.	9-Methyl-Z,Z-10,12- hexadecadien-1-ol acetate	29.423	2029	$C_{19}H_{34}O_2$	1.21
21.	9,12-Octadecadienoyl chloride, (Z,Z)-	29.481	2139	$C_{18}H_{31}CLO$	4.61
22.	Pentadecanoic acid, 2-hydroxy-(hydroxymethyl) ethyl ester	29.727	2399	$C_{18}H_{36}O_4$	1.40
23.	2,6-Octadiene,1-(1-ethoxyethoxy)-3,7- dimethyl-	30.829	1471	$C_{14}H_{26}O_2$	0.44
24.	7,11-Hexadecadienal	31.104	1816	$C_{16}H_{28}O$	5.52
25.	Cholesta-8,24-dien-3-ol,4-meth,(3.beta.,4.alpha.)-	31.452	2735	$C_{28}H_{46}O$	8.18
26.	(.+/-)-.alpha.-Tocopherol acetate	33.848	3308	$C_{31}H_{52}O_3$	8.10

At this point, the efficacy of the extract in decreasing the level of post-prandial glucose will show high potency for diabetic patients. The linear equation used to determine the dose and time needed to the action is presented in Figure 3.

Toxicological profile

The ethanolic extract of *C. grandis* L did not show any toxic signs or mortality at doses of 0.5 and 1 ml, but less movement and drowsiness, while dose 1.5 ml developed the actions to death by 80% of the experimental population.

GC-MS analysis

The chemical constituent's response from the hypoglycemic effect of the plant extract were found n-Hexadecenoic acid, beside of the oleic acid and the dietary palmitic acid: 9-Octadecanoic acid; Glycerol 1-palmitate; Cholesta-8,24-dien-3-ol; phytol exerts anti-hyperglycemic actions by different mechanisms with range of percentages in the formula was being used in the experimental mice exert hyperglycemic state as shown in Table 2, Figure 4, and Figure 5.

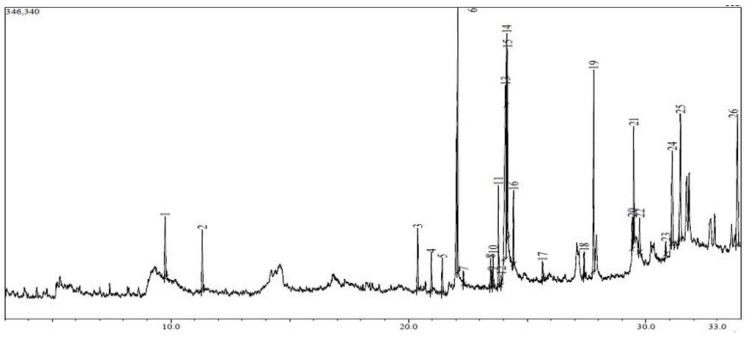

Figure 4: Chromatogram of GC-MS analysis of *C. grandis* leaf extract.

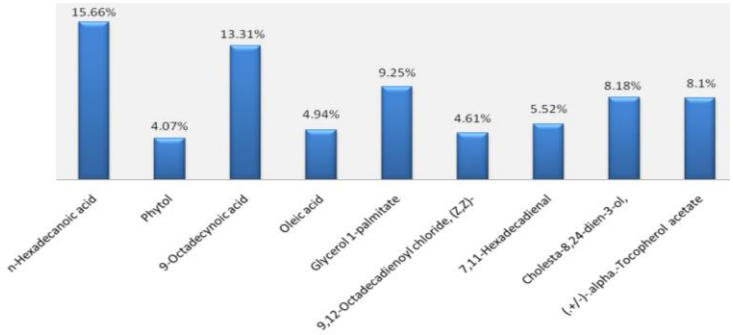

Figure 5: Quantitative detection of *C. grandis* leaves extract using LC/GC-MS.

DISCUSSION

The initial phytochemical screening of *C. grandis* indicated the existence of different chemical constituents as shown in the table and when compared with previous studies, *C. grandis* was found to contain alkaloids, the possible reason that it wasn't detected that it's may found in trace amount in the extract, reagents differences or variation of plant origin[20,21]. A study conducted by Keerthi *et al.*, reported that saponins and flavonoids are responsible for the anti-diabetic activity which is both presented in the extract[19]. On another hand, triterpenes, by blocking many pathways linked to diabetes and its consequences, can be effective treatments for diabetic retinopathy, neuropathy, nephropathy, and poor wound healing[22]. Through their beneficial effects on blood glucose levels, glucose absorption, insulin secretion, and immune function modulation, flavonoids and flavonoids-rich may lower the incidence of diabetes mellitus[23]. A statistically significant rise in serum insulin levels accompanied by a decline in the proportion of glycosylated hemoglobin due to the use of *C. grandis* plant extract with a P-value less than 0.05, correlates to the results of the current research[24]. n-Hexadecanoic acid, palmitic acid, and oleic acid elicit beneficial effects on insulin sensitivity[26,27,28].

9-Octadecanoic acid and (.+/-.)-.alpha.-Tocopherolacetate exert beneficial antioxidant activity[29,30]. Phytol exerts anti-hyperglycemia actions by delaying the digestion of lipids and carbohydrates[31]. The total dose needs approximately an hour to be fully eliminated from the blood and another dose should be given after this time to insure consistency of the response, instead of the constituent of the plant extract that is responsible for the hypoglycemic effect needing to be more concentrated in the dose given and the prediction of the exact mechanism of action also should be investigated by using multiple compartmental model according to the previous study[3]. The free radical scavenging and antioxidant activity of the ethanolic leaf extract is possibly because the fractions contain flavonoid and phenolic components.

A study revealed that one of the key medicinal uses of *C. grandis* is its ability to regulate blood sugar levels[25]. It contains compounds that have been found to possess anti-diabetic properties, making it a valuable natural remedy for individuals with diabetes or those at risk of acquiring the condition. Studies have demonstrated that the plant's extracts can enhance insulin sensitivity and assist reduce blood glucose levels, thus aiding in the management of diabetes. The current study's findings suggest that *C. grandis* leaves may be a good source of naturally occurring antioxidants that help control blood sugar levels.

Limitations of the study

This study is not without limitations, first, the study population for the *in-vivo* testing is small due to the unavailability, besides the plant species only one was being used and not available in the local market. Moreover, dosing preparation in suitable pharmaceutical products and testing of its activity is required.

CONCLUSIONS

The phytochemical screening and GC-MS revealed that the ethanolic extract is rich in phytoconstituents. The antihyperglycemic effect of the extract showed comparable results to that obtained by several previous studies. The antioxidant activity complies with literature review. Generally; the findings of this study complied with the traditional medicine of this plant and

it could be a solid ground for developing new drugs in the future.

AUTHOR'S CONTRIBUTION

Ahmed RM: literature search, conceptualization, methodology. **Widdatallah MO:** supervision, writing (original) draft, review. **Alrasheid AA:** supervision, writing draft, review, and editing. **Widatallah HA:** conceptualization, methodology. **Alkhawad AO:** supervision, review and editing. All authors revised the article and approved the final version.

DATA AVAILABILITY

The data will be available to anyone upon request from the corresponding author.

CONFLICT OF INTEREST

None to declare.

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