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Research article

Hypoglycemic Effect of 24-Methylencycloartan-3-one Isolated from *Prosopis juliflora* Pods in Alloxan Induced Diabetic Rabbits

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ABSTRACT

Prosopis juliflora is herbal plant uses in different countries to treat diabetes mellitus. In current study, oil extract was extracted from *P. juliflora* pods. Qualitative preliminary tests showed the extract contained terpenoids. The oil compound was isolated purified and identified by thin layer chromatography (TLC), gas chromatography-mass (GC-MS), nuclear magnetic resonance (H1-NMR and C13-NMR) and infra red (IR) spectroscopy. It was found that oils extract contain 24-Methylencycloartan-3-one. Hypoglycemic effect for this compound was checked in alloxan induced fasted diabetic rabbits. The dose of 0.3 gm/kg of terpenoidic at different time intervals (2,4,6 and 24 h) reduced blood glucose significantly with values 321.42, 294.60, 251.40 and 172.30 mg /100 ml, respectively. The toxicity study has showed that the 24-Methyl cycloartan compound has no toxic effect on red blood cells; therefore we suggest that this compound can be successfully and safely use to treat diabetes mellitus instead of insulin

Keywords: *Prosopis juliflora*, **terpenoids, diabetes mellitus, glucose, Alloxan**

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INTRODUCTION

Diabetes mellitus is characterized by an increased concentration of blood glucose due to disorder in carbohydrates metabolism and failure secretion of insulin. These metabolic disturbances result in acute and long-term diabetic complications, which are responsible for premature death and disability [1].Wild et al. (2009) reported that in the year 2000, there were about 171 million diabetes mellitus cases worldwide in patients ages 20 years or more, 17 million of these cases were found in the United States of America, making the US the third highest country in the prevalence of diabetes mellitus after India and China [2]. In 2010, International Diabetes Federation reported a diabetes prevalence of 12.3% in the USA

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and 7.8% in Iraq. However, in 2008, it was reported that the diabetes prevalence of 7.43% in Basrah [3], in 2011 Al-Windi and Ali reported a prevalence of 6.8% in Sulaimani, Iraq [4]. The using of 5-6 herbal drugs that can reduce dose of insulin, it would be positively contributed in the treatment of diabetes [5]. Many herbal drugs have been recommended for the treatment of diabetes mellitus, medicinal plants have the advantage of having no side effects [6]. Traditional plant treatments have been used throughout the world for the therapy of diabetes mellitus. History showed that medicinal plants have been used in traditional healing around the world for a long time to treat diabetes; this is because such herbal plants have hypoglycemic properties and other benefical properties, as reported in scientific literatures [7].

The medicinal value of plants can be observed from the chemical agents they possess, which may alter certain physiological actions in the human body. The most important of these biochemically active compounds of plants are terpenes, alkaloids, flavonoids and phenolic compounds. Terpenes are used as insecticides and their harmacological properties include antibacterial, antifungal, antihelmintic, antimalarial and molluscicidal [8]. Triterpenoids pentacyclic triterpenoids are known to exhibit a wide range of pharmacological and other biochemical activities, which include antioxidant, anti-allergic, anti-inflammatory, anti-tumour gastro protective, antibacterial, and hepatoprotective effects [9].

P. juliflora commonly known as mesquite, vilaytibabul, vilayti kikar belongs to family Leguminosae sub family, Mimosoidae. It is a large shrub to a small evergreen tree. Usually tree attains a height of 9–12 m and a girth of 90 cm. Under favorable conditions the tree attains a height of 18 m [10].

Extracts of *P. juliflora* seeds and leaves have several in vitro pharmacological effects such as anti-bacterial, anti-fungal and anti-inflammatory properties [11]. These properties have been attributed to piperidine alkaloids. A number of chemical compounds have also been reported from this plant, the most common of these being steroids, tannins, leucoanthocyanidin and ellagic acid glycosides. A new monocyclic diketone, prosopidione, and two alkaloids, namely, juliprosinene and juliflorinine, were isolated from the leaves [12].

Many anti-diabetic plants have a necessary role to treat diabetes mellitus because they have a strong action to decrease hyperglycemia. That is why; in current study this plant was used to evaluate the hypoglycemic effect of this plant. In present study we isolated terpenes from this plant and hypoglycemic activity of this chemical (terpenoids) was evaluated in fasted alloxan induced diabetic rabbits. Moreover, the extracted chemical was identified, purified by many technologies.

MATERIALS AND METHODS

Studied plant

Mature pods of *P. juliflora* were collected in May 2013 from Abu Al-khasseb near Shat Al- Arab region, Basra, Iraq. The plant was classified in the biology department, college of education for pure science, University of Basra. Fifty gram of all parts of plant were dried at 25°C and then ground by a blender (Rotel coffee grinder type 24), and kept in plastic containers at room temperature until time of experiment.

Isolation of oils from *P. juliflora* pods

Fifty grams of dried ground pods of *P. juliflora* were continuously extracted by soxhlet apparatus by 500 ml of hexane for 24 h, and then the solvent was removed under vacuum (Puchi Rotara-RE, Rotary evaporator) to afford 2.932 gm of viscous oil [13,14].

Qualitative and quantitative analysis of triterpenes extracted from *P. juliflora* pods

Triterpenes test

1 ml of concentrated of sulphuric acid was added to 1 ml of oils dissolved in1 ml of chloroform the purple-red formed indicates the presence of triterpenes [13].

Experimental Animals

Rabbits weighing 1.5-2.0 Kg were purchased from central animal house of Basra University, Basra, Iraq. Animals were kept in clean polypropylene cages and fed standard antibiotic free diet. All animals were kept in fasting for 24 h before starting the experiments.

Alloxan induced diabetic rabbits

The rabbits were dosed with three injections of alloxan monohydrate dissolved in sterile normal saline. After this process, rabbits became in hyperglycemia case. Prepared alloxan was used immediately after preparation and was administrated at period of 48 hs in a dose equal to 150 mg/Kg body weight of rabbits and injected via marginal ear vein under light by 1 ml syringe [15] and then 20 % of glucose dissolved in drinking water was given to rabbits orally. They were kept in fasting for 18 hs after seven days from last administration. Glucose concentration was measured in blood of alloxan-induced diabetic rabbits by using glucose oxidase peroxidase enzymatic colorimetric GOD-PAP method by using meaning strips [15].

Effect of terpenoids on glucose level in alloxan–induced diabetic rabbits

Twelve fasted hyperglycemic rabbits were divided into two equal groups. The first was given 3 ml of normal saline and considered as a control group, while the second group was given 0.3 gm/kg body weight of

terpenoids extract dissolved in 3 ml of sun flower, which was considered as treatment group (test group). Blood samples were collected at different time intervals (0, 2, 4, 6 and 24 h). The glucose concentrations were measured at each time point [15].

Isolation and Purification of Compounds

For the isolation process, the techniques of column chromatography and thin layer chromatography (TLC) were used in chemistry department laboratories, Faculty of Engineering and Physical Sciences at the University of Surrey in United Kingdom. Different sized columns were used for column chromatography, ranging from 2-6 cm diameter depending on the purification stage and the amount of the sample available. The separation of compounds was carried out using thin layer chromatographic analysis and size-exclusion column chromatography over Sephadex using (CH2Cl2: Hexane, 1:1) as the mobile phase. TLC analysis was carried out in 0.2 mm silica gel, aluminium-backed plates (Merck Art.5554).

Identification spectral of terpenoids

Various spectrophotometric techniques including Nuclear magnetic resonance spectroscopy (NMR), Fourier transform infrared spectrum (FTIR), and Mass Spectrum (MS) analysis were used to identify the chemical compounds of *P. juliflora* pods extract [16].

Nuclear magnetic resonance spectroscopy (NMR)

Nuclear magnetic resonance spectrum was recorded on a 500 MHz Bruker AVANCE NMR spectrophotometer in the chemistry department at University of Surrey in United Kingdom. The spectrum was recorded in either deuterated chloroform (CDCl3) or deuterated methanol (CD3OD)

Mass Spectrum (MS)

Low resolution electron impact mass spectra were acquired by using a Hewlett Packard G1800A GCD instrument spectrum in chemistry department at University of Surrey, United Kingdom.

Fourier transform infrared spectrum (FTIR)

Infrared spectrum was recorded using a Perkin-Elmer (2000 FTIR) spectrophotometer. The samples were dissolved in chloroform and analyzed using NaCl plates.

Determination of cellular toxicity for oil extract and for 24-Methylencycloartan-3-one compounds isolated from *P. juliflora*

Determine the cellular toxicity of some of the oil extract and 24-Methylencycloartan-3-one extracted from *P. juliflora*. 2000 mg of extract dissolved in 10ml normal saline and then serial dilutions (1:1, 1:10, 1:100 and 1:1000 v/v) were prepared. The control was contained normal saline. 0.8 ml of each concentration was put in a sterile test tube (Eppendorf tubes). The tubes contained anti-clotting substance. 0.2 ml of fresh human blood was added to each tube to 1 ml as a final volume. The tubes were incubated at 37 ^{o}C for 30 min the tubes were centrifuged for 5 min [17].

Statistical Analysis

The statistical analysis was carried out for all experiments of hyperglycemic rabbits by one way ANOVA method of variance analysis using SPSS [14] for testing presence of significant differences between control and treatment means.

RESULTS

Percentages of *P. juliflora* pods Extracts

Percentages of *P. julifloa* pods extracts were calculated as illustrated in **table 1.**

Table 1. Percentage of *P. juliflora* pods extracts

No	Extract type	Percentage	Physical nature
1.	Oil	6.34%	Viscous liquid

Qualitative analysis of terpenoids extract isolated from *P. juliflora* pods

Qualitative analysis of terpenoids isolated from *P. juliflora* pods was shown in **table 2.**

Table 2. Qualitative analysis of terpenoid isolated from oil extract of *P. juliflora* pods

Reagents	**Oil extract**	**Results**
Libermann-Burchard	++	Triterpenes are present
Salkoviski	+++	Triterpenoids are present

Effect of oral administration of terpenoids on glucose level in alloxan induced diabetic rabbits

The effect of terpenoids compounds extract of *P. juliflora* pods on blood glucose level in hyperglycemic rabbits at different time points after oral administration was shown in **table 3**. It was found that the extract decreased significantly the glucose level at 4 h ($P<0.05$) and at 6 h ($P<0.01$). The highest significant decrease was found at 24 h ($P<0.001$).

Thin Layer Chromatography (TLC)

TLC results of terpenoids extract showed the presence of one spot, this indicate presence one terpenoid compound. Therefore, it was separated by TLC, using

Table 3. Effect of oral administration of terpenoids extract at *P.juliflora leaves* on blood glucose concentration in hyperglycemic Rabbits. Blood glucose concentrations were presented as mean ± S.E.M.

Extract and dose (gm/kg)	No	Blood glucose concentration (mg/100 ml)				
		0 h	2 h	4 h	6 h	24 h
Control 3m l sun flower	6	350.0 ± 7.23	360.00 ± 3.97	353.35 ± 3.21	342.8 ± 2.96	321.68 ± 3.57
0.3 g/kg (Oils)		342.3 ±5.04	321.42* ± 3.09	294.6** ± 7.40	251.4** ± 10.17	172.30*** ± 3.10

*, P<0.05; **, P<0.01; ***, P<0.001

No: number of rabbits in each group.

100% DCM. The sample was lined across the TLC plate, 1 cm from the bottom of the plates. The plates were then placed in chromatography tanks and left to develop in the desired solvent system. The compounds of interest were successfully separated and examined under UV light and anisaldehyde spray regent and heating.

Spectral identification of terpenoidic compound GC-Mass

The Mass spectrum was recorded for compound isolated from the pods of *P. juliflora* as shown in **fig 1,** which indicates the abundance and m/z Peaks.

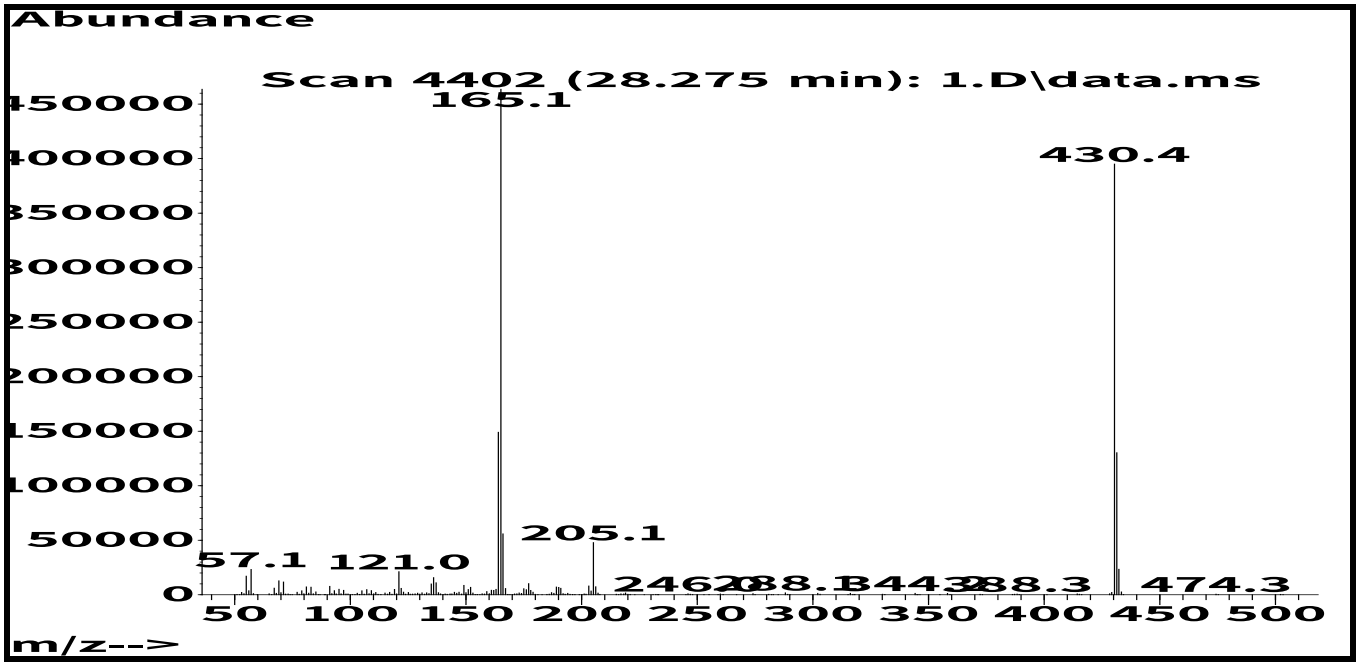

Fig 1. Mass spectrum for 24-Methylencycloartan-3-one in $CDCl_3$.

FTIR spectrum

Infrared spectrum was recorded for the compound isolated from the leaves of *P. juliflora* and shown in **fig 2** and **table 4**, which showed the intensity of the absorption peaks and structural groups.

Nuclear magnetic resonance spectroscopy (NMR)

1H NMR, 13C NMR spectra were recorded 24-Methylencycloartan-3-one compound isolated from leaves of *P. juliflora* **(figs 3, 4, 5** and **6)**. **Table 5** showed the chemicals shifts for hydrogen groups and carbons atoms.

Table 4. The intensity of the absorption packets and wave number.

Wave number Cm $^{-1}$	Group	Vibration Type	Intensity
2847.9	CH	Stretching	Strong
2915.9	CH	Stretching	Strong
1727.0	C=O	Stretching	Strong
1122.9	C-O	Stretching	Strong
983.5	CH	Bending	Strong
774.0	CH_2	Bending	Weak

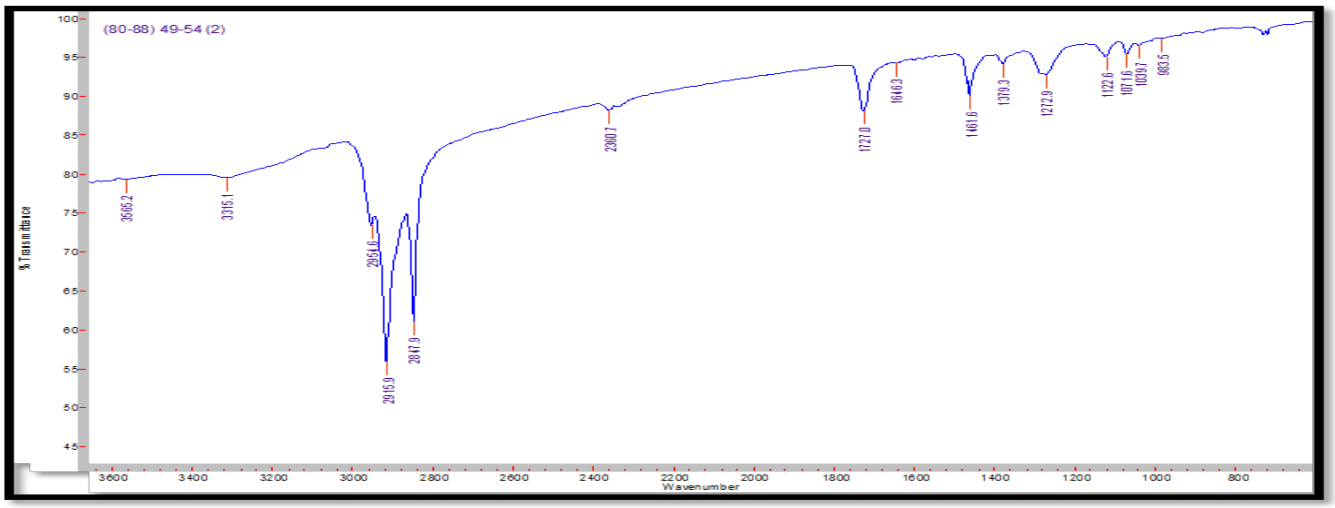

Fig 2. FTIR spectrum for 24-Methylencycloartan-3-one $CDCl_3$.

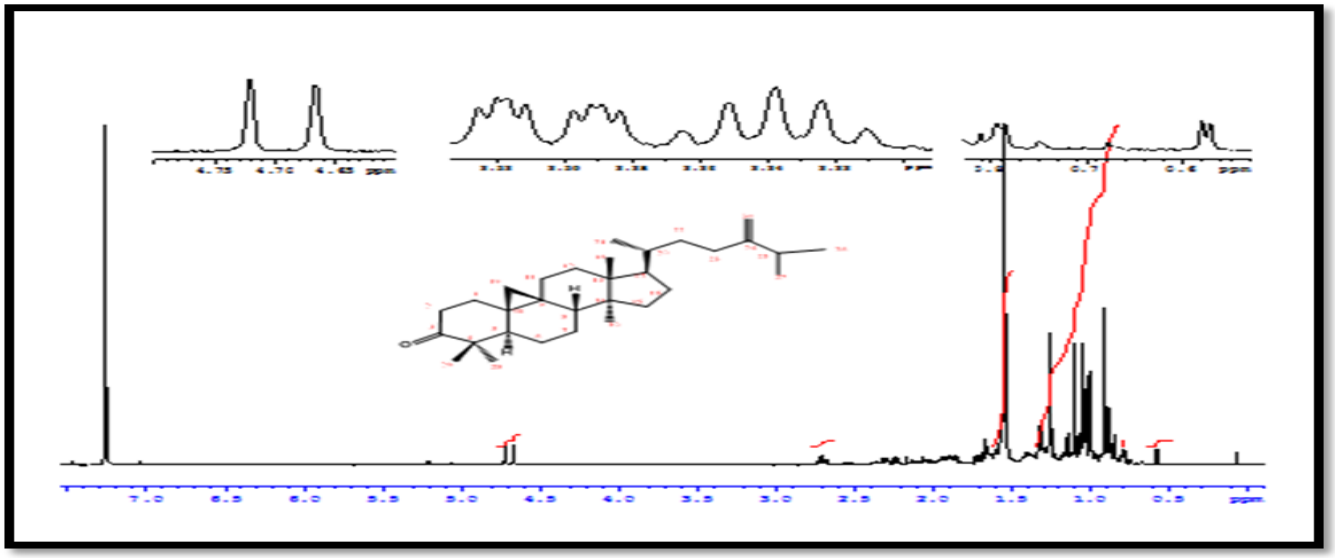

Fig. 3. [1] NMR spectrum for compound **24-Methylencycloartan-3-one** in $CDCl_3$.

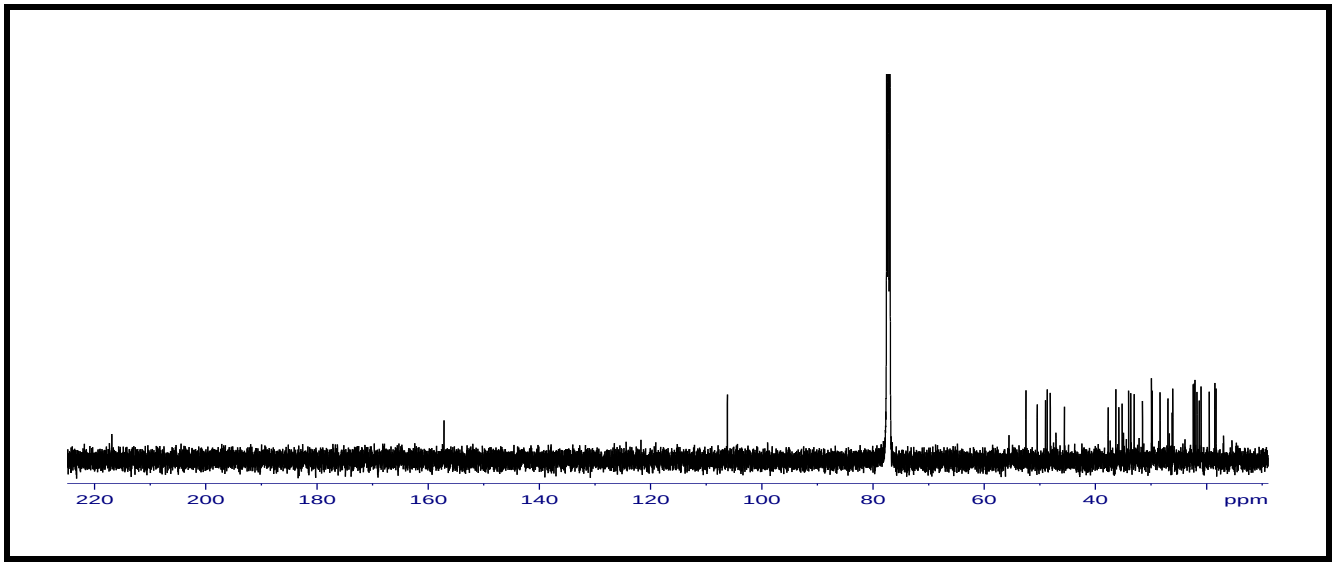

Fig.4 C^{13} NMR spectrum for compound **24-Methylencycloartan-3-one** in $CDCl_3$.

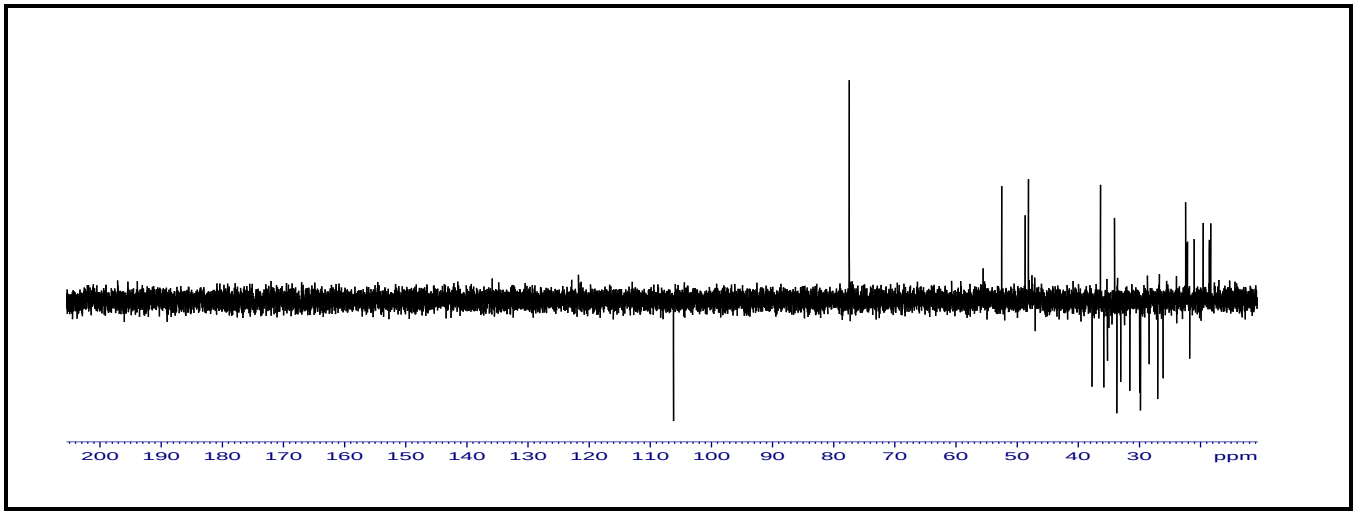

Fig.5 DEPT spectrum for compound **24-Methylencycloartan-3-one** in $CDCl_3$

Table 5. NMR data for compound 24-Methylencycloartan-3-one in $CDCl_3$compared against reference values.

No	^{1}H NMR (500 MHz)	^{13}C NMR (125 MHz), in $CDCl_3$ (Alves *et al.*, 2000)	^{13}C NMR (125 MHz)
1	1.87 2H, Mm	33.4	33.7 CH2
2	2.30 2H, ddd, m	37.4	37.7 CH2
3	-	217.5	216.9C
4	-	50.2	50.5C
5	1.70 H, m	48.4	48.7CH
6	1.54 H, m 0.95 H, m	21.5	21.7 CH2
7	1.12 H, m 1.40 H, m	25.8	26.1 CH2
8	1.62 H, m	47.8	48.13 CH
9	-	21.0	21.3 C
10	-	25.9	26.2 C
11	2.05 2H , m	26.7	27.0CH2
12	1.67 2H , m	32.7	33.0CH2
13	-	45.2	45.6 C
14	-	48.6	49 C
15	1.35 2 H, m	32.2	35.8 CH2
16	1.33 2H, m	28.1	28.4 CH2
17	1,62 H, m	52.2	52.5 CH
18	1.00 3H, S	18.5	18.3 CH3
19	0.80 3H, S, d	29.6	29.8CH3
20	1.40 H, m	36.0	36.3 CH
21	0.91 3H	18.2	18.5 CH3
22	1.14 H, m 1.57 H, m	3.01	34.1 CH2
23	2.13 H, m 1.90 H, m	31.2	31.5 CH2
24	-	157.5	157.2C
25	2.24 H, Septet	33.7	34.0 CH
26	1.04 3H, d	22.0	22.1 CH3
27	1.02 3H, d, m	22.8	22.5 CH3
28	1.06 3H,s	20.7	22.5 CH3
29	1.08 3H,s	19.3	19.3 CH3
30	4.67 H bro. 4.20 H bro.	103.9	106.2 CH2

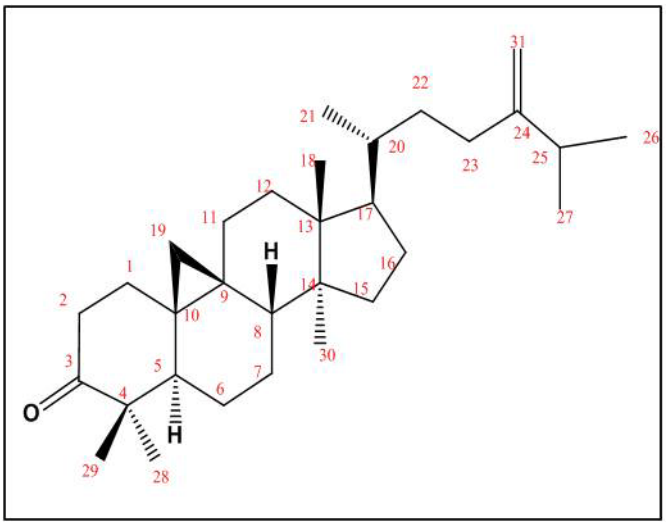

Fig. 6 chemical structure of 24-Methylencycloartan-3-one compound

Toxcity of extractions and compound isolated from *P. juliflora* pods

Table 6 shows the toxicity of extract and isolated compound obtained from the pods P. juliflora. The result showed there is no toxicity effect against human red blood cells at each concentration.

Table 6. Hemolysis of RBCs at different concencentrations of plant extracts

	Hemolysis			
Extraction type	100 g/ml	20 mg/ml	2 mg/ml	0.2mg/ml
Oil pods	–	–	–	–
24-Methylencycloartan-3-one	–	–	–	–

DISCUSSION

It was observed from **tables 3** that terpenoids extracted from pods of *P. juliflora* decreased blood glucose concentrations in hyperg-lycemic rabbits significantly because of presence of terpenes in this extract, where more studies indicated that plants and herbs containing terpenes compounds have high decreasing effect of hyperglycemic [17]. Furthermore terpenes has an anti-oxidative activity to capture free radicals, therefore these chemical compounds protect the insulin hormone from free radicals and then decreasing oxidation of this hormone [18]. Many active ingredients extracted from herbal plants possess therapeutic values, i.e. hypoglycemic activity, antioxidant action, etc and they are yet to be discovered. Generally, leaves are the favorable storage site for desired compounds and more than 35% of the plants extracts for diabetes treatment can be obtained from these parts [19]. The study of Singh, 2012 showed that leaf and pod to be the richest source of plant metabolite, followed by flower, root and stem. Phytochemical analysis of the extracts revealed presence of tannins, phenolics, flavonoids, alkaloids, terpenes and steroids in most parts of *P. juliflora* [20]. 24-methylencycloartan-3-one was isolated as a white oily material from the dichloromethane extract of the stem bark of T. sinensis and was found to be 24-methylencycloartan-3-one. It has been isolated previously from many species such as Cedrela odorata grafted on T. ciliate (Meliaceae) [21], *Euphorbia helioscopia* (Euphorbiaceae) [22], *Krameria tomentosa* (Krameriaceae) [23] *Larix kaempferi* (Pinaceae) [24] and Microsorium fortune Moore (Polypodiaceae) [25].

The GC-MS of this compound gave a [M+H]+ ion peak at m/z439.3615, calculated for [M+H]+439.3940, which indicated a molecular formula of C31H50O. A double bond equivalence of seven was calculated for this compound. The FTIR spectrum showed absorptions bands at 2817.0 and 2815.9 cm-1 for C-H stretches and 1727.0cm-1 for the carbonyl stretch of a ketone group [26]. 13C NMR spectrum for (24-methylencyc-loartan-3-one) showed a resonance at δc 216.9for ketone group at C-3. Two H-2 resonances appeared more downfield at δH 2.72 and 2.30 in compound (24-methylencycloartan-3-one. A compareson of the spectroscopic data for compound (24-methylencyclo-artan-3-one) with that found in the literature [22- 24].

From present study it can be concluded that terpenoid decreased significantly blood glucose concentrations in hyperglycemic rabbits. The active compound in the oil extract was 24-Methylencycloartan-3-one and this compound is not toxic for eukaryotic cells in vitro; that is why this compound can be used safely to reduce the level of glucose in blood circulation of human and animal as well.

Conflict of interest:

The authors declare that they have no conflict of interests.

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