Biological evaluation of *Cornulaca monacantha* Del

Mohamed A. Ashour[1,2]*, Bader Alsuwayt[3]

[1] Department of Phytochemistry and Natural Products, Faculty of Pharmacy, Northern Border University, Rafha- 91911, P.O. BOX 840, Kingdom of Saudi Arabia.
[2]Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Nasr city, Egypt.
[3] Department of pharmacology, Faculty of Pharmacy, Northern Border University, Rafha- 91911, P.O. BOX 840, Kingdom of Saudi Arabia.

ABSTRACT

C. monacantha has been previously investigated for its phytochemical compounds where 13 triterpenoidal saponins, 6 flavonoid derivatives and 4 Gallotannins have been reported. The aerial part of *C. monacantha* is known in folk medicine, especially in desert and rural areas of North Africa and Middle East where this plant is growing, for the treatment of jaundice and other liver diseases, it has also purgative effect and increase the milk supply for camels. The purpose of present study includes biological investigation of different *C. monacantha* extracts. The total alcoholic extract of *C. monacantha* was fractionated into four fractions; ether, ethyl acetate, saponins and acetone fractions. Different *C. monacantha* fractions have been investigated for the first time in the present study regarding their hepatoprotective, antidiabetic, anti-inflammatory, antioxidant, antimicrobial and cytotoxic activities. The most active fraction was ethyl acetate fraction while the saponins fraction showed the lowest effect in all biological screening tests. Further investigation of the ethyl acetate fraction is recommended to explain and identify the correlation between the phytochemical constituents of ethyl acetate fraction of *C. monacantha* and the biological activities.

Key Words: *Cornulaca monacantha, hepatoprotective, antidiabetic, anti-inflammatory, antimicrobial and cytotoxic activities.*

eIJPPR 2019; 9(5):71-75

HOW TO CITE THIS ARTICLE: Mohamed A. Ashour, Bader Alsuwayt (2019). "Biological evaluation of *Cornulaca monacantha* Del", International Journal of Pharmaceutical and Phytopharmacological Research, 9(5), pp.71-75.

INTRODUCTION

Cornulaca monacantha del. (in Arabic known as "Had") is wild plant spread in the Mediterranean region and Arabia [1]. Preliminary phytochemical screening of *C. monacantha* revealed the presence of alkaloids, coumarin, triterpenoid saponins, flavonoids, polyphenolic compounds and tannins [2]. *C. monacantha* has been previously investigated for its phytochemical compounds where 13 triterpenoidal saponins [2-4], 6 flavonoid derivatives [5, 6] and 4 Gallotannins have been reported [6]. The aerial part of *Cornulaca monacantha* is known in folk medicine for the treatment of liver diseases and scabies [1]. It has also purgative effect and increases the milk supply for camels [1] and reported to has molluscidal activities [2]. The present study deals with biological investigation of different *C. monacantha* extracts for their hepatoprotective, antidiabetic, anti-inflammatory, antimicrobial and cytotoxic activities.

MATERIALS AND METHODS:

Plant Material:

1.5 kg powdered air dried aerial part of *Cornulaca monacantha* were extracted with ethanol 70 %, the ethanol extract were dried under vacuum. The extract was subjected to sequential fractionation using diethyl ether (27.6 g) then ethyl acetate (19.8 g). The remaining extract was treated with acetone where the saponin fraction was precipitated out (45 g.), in addition to the acetone-soluble fraction (55 g.). Four *C. monacantha* fractions were subjected to *in vitro* biological screening procedures including: anti-inflammatory, antioxidant, antimicrobial and cytotoxic activities in addition to antidiabetic activity

Corresponding author: Mohamed A. Ashour
Address: Associate Professor, Department of Phytochemistry and Natural Products, Faculty of Pharmacy, Northern Border University, Rafha-91911, P.O. BOX 840, KSA.
E-mail: ✉ ashourmohamed08 @ yahoo.com
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Received: 20 July 2019; **Revised:** 23 November 2019; **Accepted:** 08 December 2019

using two enzymes (α-glucosidase and α-amylase enzymes inhibition assays).

Phytochemical Screening:

The total extract of *C. monacantha* was subjected to phytochemical screening using phytochemical screening procedure [7-9].

Hepatoprotective Activities:

The experiment is based on the exposure of HepG2 cells to toxicant containing 1% CCl_4 along with /without tested sample of non toxic concentrations or the medium alone (control). At the end of the period, cell viability was assessed by the MTT reduction assay [10, 11].

The experimental groups were as follows: Group 1: Control, untreated HepG2 cells; Group 2: HepG2 cells treated with 1% CCl_4; Group 3: HepG2 cells treated with 1% CCl_4 and tested compound; Group 4: HepG2 cells treated with 1% CCl_4 and silymarin as standard drug. Each treatment was repeated four times (i.e. 4 wells for each treatment in the 96-well microtitre plate). After 24 h incubation, the medium was removed and 50 µl of 3-(4,5-dimethylthiazol-2-yl)‑2,5‑diphenyltetrazolium bromide (MTT) (5 mg/ml; Sigma, St. Louis, CA, USA) was added to each well. The plates were gently shaken then incubated in the dark at 37˚C for an additional 4 h in 5% CO_2 atmosphere. The reaction was stopped by the addition of 150 µl DMSO (Sigma) and the absorbance of samples at 570 nm was measured with a microplate reader (SunRise, Tecan, USA). The percentage of viability was calculated as [(ODt/ODc)]x100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. Hepatoprotective Percent = % Viability of treatment group – % Viability of negative control**.**

Antioxidant Activity:

The antioxidant activities of *C monacantha* fractions were determined using the DPPH free radical scavenging assay [12] in triplicate and average values were considered.

Cytotoxic Activity:

Different *C. monacantha* fractions were investigated for their cytotoxic activities using two mammalian cancer cell lines: HepG-2 cells (human hepatocellular cancer cell line) and A-549 cells (human lung carcinoma) which were obtained from VACSERA Tissue Culture Unit (Cairo, Egypt). The test for Cytotoxicity evaluation of different *C. monacantha* fractions at concentrations ranging from 0.0 - 500 µg/ml was done using viability assay (MTT assay) procedure [12]

Antimicrobial Activity:

The evaluation of antimicrobial activity of different *C. monacantha* fractions were carried out using well diffusion method [12]. Two fungal strains *Candida albicans* RCMB 005003 (1) ATCC 10231 and *Aspergillus fumigatus* (RCMB 002008), two gram-negative bacteria *Proteus vulgaris* RCMB 004 (1) ATCC 13315 and *Escherichia coli* (RCMB 010052) ATCC 25955; and two gram-positive bacteria *Staphylococcus aureus* (RCMB010010), and *Bacillus subtilis* RCMB 015 (1) NRRL B-543, were used as test microorganisms. The diffusion agar technique was applied, well diameter: 6.0 mm (100 µl was tested), where ketoconazole (100 µg/ml) and gentamycin (4µg/ml) were used as positive control for antifungal and antibacterial activities respectively, the samples were tested at 1.0 mg/ml concentration.

Antidiabetic activities:

In-vitro antidiabetic evaluation of the *C. monacantha* was carried out using both α-amylase and α-glucosidase inhibitory assays.

α-amylase inhibitory assay:

It was conducted using a modified procedure [13], briefly, A total of 250 µL of each fractions (0.0–1000 µg/mL) was placed in a test tube and 250 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing 1.0 M of α-amylase solution was added. This solution was pre-incubated at 25°C for 10 min; then, 250 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added at timed intervals and then incubated at 25°C for 10 min. The reaction was terminated by adding 500 µL of DNS reagent. The tubes were then incubated in boiling water for 5 min and cooled to room temperature. The reaction mixture was then diluted with 5 mL distilled water and the absorbance was measured at 540 nm. The –ve control and standard (+ve control) were tested by the same procedure using distilled water and acarbose (0.0–1000 µg/mL) respectively instead of *C. monacantha* fractions.

α-glucosidase inhibitory assay:

The effect of the plant fractions on α-glucosidase activity was determined. [13] In brief, different concentrations (0.0–1 mg/mL) of *C. monacantha* fractions were prepared in distilled water. Then, 50 µL from the stock solution was mixed with 100 µL of 0.1 M phosphate buffer (pH 6.9) containing 1.0 M of α-glucosidase solution. The mixtures were then incubated in 96-well plates at 25°C for 10 min. Following this, 50 µL of 5 mM p-nitrophenyl-α-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25°C for 5 min. The enzyme activities were determined by measuring the absorbance of the reaction mixtures at 540 nm. The –ve control and standard (+ve control) was tested by the same

procedure using distilled water and acarbose (0.0–1 mg/mL) respectively instead of *C. monacantha* fractions.
The experiments were conducted in triplicate and the α-amylase and α-glucosidase inhibitory activities were expressed as % inhibition using the expression:
% Inhibition = $[(A_{control} - A_{extract})/A_{control}]\times100$,
where $A_{control}$ and $A_{extract}$ are the absorbances of the control and fractions, respectively. Concentrations of fractions resulting in 50% inhibition of enzyme activity (IC_{50}) were determined graphically.

Anti-arthritic effect.

Evaluation of anti-arthritic effect of *C. monacantha* fractions on inhibition of protein denaturation was conducted using bovine serum albumin (BSA) procedure [14].
The reaction mixture (0.5 ml) contained 0.45 ml BSA (5% aqueous solution) and 0.05 ml of different concentrations (0.0 – 1000 µg/ml) of *C. monacantha* fractions and Diclofenac sodium (reference drug), correspondingly. Each solution was adjusted to pH 6.3 by 1 N HCl. The samples were incubated at 37 °C for 20 min and heated at 57 °C for 30 min. Then phosphate buffer (2.5 ml) was added and absorbance was measured at 660 nm via spectrophotometer. For test control 0.05 ml distilled water was used instead of fractions while product control lacked BSA. The percentage inhibition of protein denaturation was obtained by the following formula:

Percentage inhibition =

$$100-\left[\frac{\text{Abs of Test Solution}-\text{Abs of Product Control}}{\text{Abs Test Control}}\right]\times100$$

Where: Abs = Absorbance

All determinations were carried out in triplicate manner and values are expressed as the mean ± SD. The IC_{50} value is defined as the concentration of inhibitor that produce 50% inhibition under the assayed conditions.

RESULTS:

phytochemical constituents of C. monacantha:

According to the phytochemical screening of the total extract of *C. monacantha*, the presence of glycosides and/or carbohydrates, sterols and/or triterpenes, saponins, flavonoids, tannins and traces of alkaloid contents were established; while the anthraquinones were not detected in the sample.

Biological activities:

Anti-arthritic activities of *C. monacantha* extracts:

Anti-arthritic potential of different *C. monacantha* fractions were evaluated *in-vitro* using protein denaturation (bovine serum albumin) method at 0 –1000 µg/ml concentrations. While the IC_{50} (µg/ml) of *C. monacantha* fractions were >1000, 951, >1000, 60.9 for acetone, saponin, ether and ethyl acetate fractions respectively; while the IC_{50} (µg/ml) of reference substance (diclofenac) was 15.12 µg/ml.

Anti-diabetic activities of *C. monacantha* extracts:

Two enzyme inhibitory assays; α-amylase inhibitory assay and α-glucosidase inhibitory assay were used to evaluate the antidiabetic potential of different *C monacantha* fractions. The IC_{50} (µg/ml) were 123.6, >1000, 485.2, 115.8 for acetone, saponin, ether and ethyl acetate fractions respectively; while the IC_{50} (µg/ml) of reference substance (acarbose) was 34.71 in case of α-amylase inhibitory assay; while IC_{50} (µg/ml) of different fractions using α-glucosidase inhibitory assay procedure were 108.5, 901.6, 437.7, 60.4 for acetone, saponin, ether and ethyl acetate fractions respectively; and the IC_{50} (µg/ml) of reference substance (acarbose) was 30.57.

Anti-oxidant activities of *C. monacantha* extracts:

Antioxidant potential of different subfractions were evaluated in vitro using the DPPH free radical scavenging assay at 0 –1000 µg/ml concentrations where IC_{50} (µg/ml) of different subfractions were 405.5, 232, 195.7, 77.1 for acetone, saponin, ether and ethyl acetate fractions respectively; while the IC_{50} (µg/ml) of reference substance (ascorbic acid) was 14.2

Hepatoprotective activities of *C. monacantha* extracts:

Hepatoprotective activity testing was done in vitro for different subfractions using HepG-2 cell line at Non cytotoxic conc. 30 µg/ml. where the percentages of hepatoprotection were 20.5 %, 10.9%, 13.7% and 24.6 % for acetone, saponin, ether and ethyl acetate fractions respectively; while the IC_{50} (µg/ml) of reference substance (silymarin) was 87%.

Cytotoxic activities of *C. monacantha* extracts:

Two mammalian cancer cell lines: HepG-2 cells (human hepatocellular cancer cell line) and A-549 cells (human lung carcinoma) were chosen for Cytotoxicity evaluation using viability assay (MTT assay) procedure. IC_{50} (µg/ml) of *C. monacantha* fractions against HepG2 cells were 237.5, 484, 111, 61.7 for acetone, saponin, ether and ethyl acetate fractions respectively, while IC_{50} (µg/ml) against A-549 cells were 216.3, >500, 101, 111 for acetone, saponin, ether and ethyl acetate fractions respectively.

Antimicrobial activities:

Different fractions of *C. monacantha* extract showed variable antimicrobial activities against the tested microorganism. However the results of antimicrobial activity testing are found in table 1.

Table 1: Antidiabetic effects of different fractions of *C. monacantha* extract

Sample code / Tested microorganisms	acetone	Saponin	ether	ethyl acetate	Control
FUNGI					Ketoconazole
Aspergillus fumigatus (RCMB 002008)	NA	NA	NA	NA	17
Candida albicans RCMB 005003 (1) ATCC 10231	NA	9	NA	13	20
Gram Positive Bacteria:					*Gentamycin*
Staphylococcus aureus (RCMB010010)	12	11	12	15	24
Bacillus subtilis RCMB 015 (1) NRRL B-543	NA	12	8	10	26
Gram Negatvie Bacteria:					*Gentamycin*
Proteus vulgaris RCMB 004 (1) ATCC 13315	NA	12	15	13	25
Escherichia coli (RCMB 010052) ATCC 25955	10	11	13	12	30

NA: inactive (no inhibition zone)

DISCUSSION:

From the obtained results it can be stated that. The ethyl acetate fraction was the most active fraction of *C. monacantha* extract, where it shows IC_{50} = 60.9 compared with >1000, 951, >1000 (µg/ml) for acetone, saponin, and ether fractions respectively in the experiment of anti-arithritic activity study. Ethyl acetate fraction shows a best antidiabetic activity of all *C. monacantha* fraction where it has IC_{50} = 115.8 (µg/ml) compared with 123.6, >1000, 485.2, (µg/ml) for acetone, saponin, and ether fractions respectively through α-amylase inhibitory assay; and IC_{50} = 60.4 (µg/ml) compared with 108.5, 901.6, 437.7 (µg/ml) for acetone, saponin, and ether fractions respectively through α-glucosidase inhibitory assay (figure 1).

The antioxidant activity of ethyl acetate fraction was also the best one among all fractions where IC_{50} was 77.1 (µg/ml) compared with 405.5, 232, 195.7 (µg/ml) for acetone, saponin, and ether fractions respectively.

Hepatoprotective activities of ethyl acetate fraction are more valuable than other fractions where it showed 24.6 % hepatoprotection compared with 20.5 %, 10.9%, and 13.7% for acetone, saponin, and ether fractions respectively.

Both ethyl acetate and ether fractions have a comparable activities(IC_{50} = 101 and 111 µg/ml respectively) against A-549 cells, while ethyl acetate showed valuable activity (IC_{50} = 61.7 µg/ml) against HepG2 cells than other fractions (figure 2).

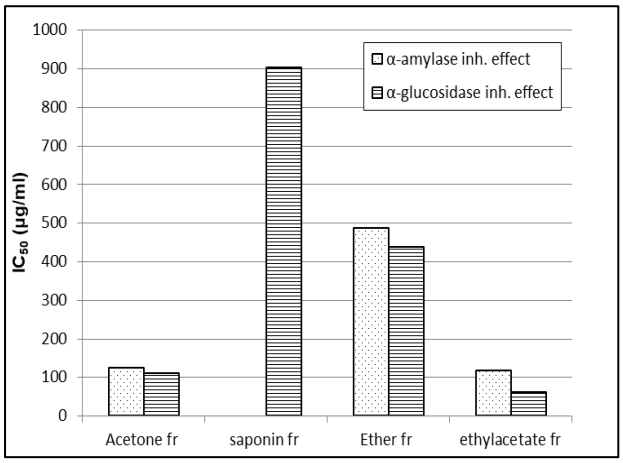

Figure 1: Antidiabetic effects of different fractions of *C. monacantha* extract

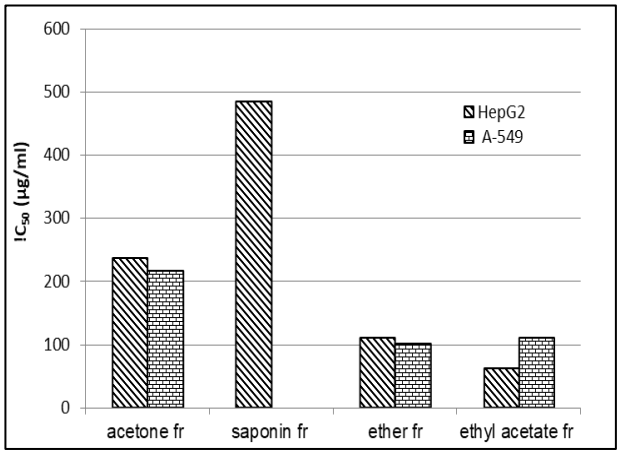

Figure 2: cytotoxic activities of different fractions of *C. monacantha* extract.

The antimicrobial activity test confirmed the mild effect of acetone fraction of *C. monacantha* it showed only inhibition zones against S. aureus and E. coli, while the most promising one was ethyl acetate fraction where it 13, 15, 10, 13, and 12 mm inhibition zones against *C. albicans*, *S. aureus*, *B. subtilis*, *P. vulgaris* and *E. coli* respectivley.

CONCLUSION

The ethyl acetate fraction showed the most promising effects among all fractions of *C. monacantha* extract. These activities may be attributed to the phytochemical compounds of ethyl acetate fraction. The phytoconstituents of ethyl acetate fraction are expected to be flavonoids and phenolic compounds which were already isolated and reported for *C. monacantha*. In the same time the triterpenoidal saponins fraction showed the lowest activities compared with other fractions of *C. monacantha* extract. The flavonoids and phenolic constituents of ethyl acetate fraction of *C. monacantha* should be isolated and re-evaluated individually for their biological activities in order to find out the direct relation between these compounds and the biological activities. The flavonoids and phenolic constituents of ethyl acetate fraction of *C. monacantha* or some of them are expected to be approved as drug leads in the near future.

ACKNOWLEDGMENT

The authors gratefully acknowledge the approval and the support of this research study by the grant No. 7632-PHM-2018-3-9-F from Deanship of scientific research at Northern Border University, its address is Arar-P.O. Box. 1321—Arar, 91431—Rafhaa international highway-Northern Border University, K.S.A.

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