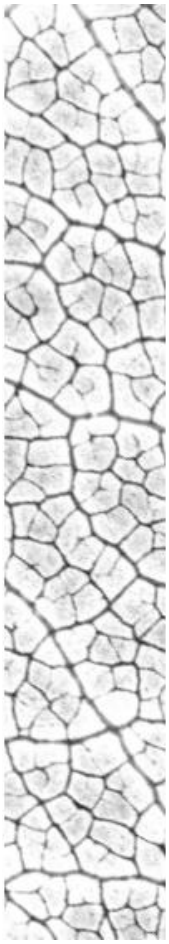

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Extraction of (3-Methyl-2-buten-1-yl β-D-glucopyranoside) of the fruit of plant Capparis spinosa and study effect on breast cancer cells

Amjed Abbawe Salih [1*], Hussein Abood Idham [2], Nuhad Khaleel Ismaeel [1,3]

[1] The College of Education for Pure Sciences at the University of Kirkuk, IRAQ
[2] Directorate General of Education Kirkuk, IRAQ
[3] The College of Sciences at the University of Diyala, IRAQ
*Corresponding author: Amjed_ch86@uokirkuk.edu.iq

Abstract
Caper plant (*Cappers Spinoza*) separate have been related with diverse biological activities inclusive anti-cancer properties, In this research, researcher used as a treatment for cancer we observe measurements of significant decrease in the level of breast cancer cells at ≥ 0.05,and identify the compound extract (***3-Methyl-2-buten-1-yl β-D-glucopyranoside***) by spectrum methods (IR,^{1}H.nmr and ^{13}C.nmr) spectroscopy.

Keywords: Caper (*Cappers Spinoza*), pyran derivatives, anti-cancer activity

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INTRODUCTION

Plants are a valuable exporter of a wide range of secondary metabolites, which are used as pharmaceuticals, agrochemicals, flavors, fragrances, colors, bio pesticides and food added ingredients (Al-Snafi, 2015). Caper (*Cappers Spinoza L*.) is native to the Mediterranean region and is also widely grown in the dry regions in west and central Asia. Its immature flower buds, unripe fruits, and shoots are consumed as foods or condiments in cooking (Tesoriere, et al. 2007). Several parts of this plant, including the flower sprouts, fruits, seeds, shoots, and bark of roots, were traditionally used as folk medicines in the treatment of disorders, such as rheumatism, stomach problems, headache, and pain in a tooth (Tlili, et al. 2010). *Cappers Spinoza* L. is the most significant economical species pertinence to the *Capper idaceae* family (Tlili N, et al. 2010). Wild species of Capp-arise are found in countries surrounding the Mediterranean basin extending to the Great Sahara in North Africa and the dry regions of Western and Central Asia. There are many important caper types of which Cappers Spinoza's the most important one. Most of these species are used as taster food in some countries (Tlili, et al. 2011). Fruits of *Cappers Spinoza*, also known as caper berries, are elliptic, ovoid to pear-shaped.In Mediterranean countries, pickled bud is a commercial commodity (Sozzi, 2008). The genus *Cappers* includes about250 species many of them distributed in the Mediterranean regions (Inocencio, et al. 2005). Breast cancer is an important public health problem universal estimates indicate that it is the second most common cancer in the world, and the most frequent cancer among women, with more than1.5 million new cases diagnosed per year (Ferlay, et al. 2015, Sarli and Ghasemi 2020). The incidence rate among women is on the height, and this is due to an earlier age of menarche, later age of first pregnancy, fewer pregnancies, shorter periods of breastfeeding and later indisposition (Ferlay, et al. 2015). Other known risk factors for breast cancer are also increasing, inclusive obesity, alcohol use and lack of physical activity (Howell et al. 2014).

Anti cancer Activity

Cancer, was described as, cell propagation which invades other tissues as well or an uncontrolled growth. The mechanism beyond tissue invading is through direct cell emigration or lymphatic system and blood. The hazard agents for cancer consist of an unhealthy diet, environmental factors, infection, radiations, chemicals and smoking (Anand et al. 2008). Hundred various kinds of cancer named by the type of cell or tissue or organ in which they start.Their intensity may be benign (earlier period) or malignant (end stage, called cancer) (Becker et al. 2009). Natural products from biological sources and plants still stay an uncondensed source and unlimited of new nutraceuticals and phytochemicals. In

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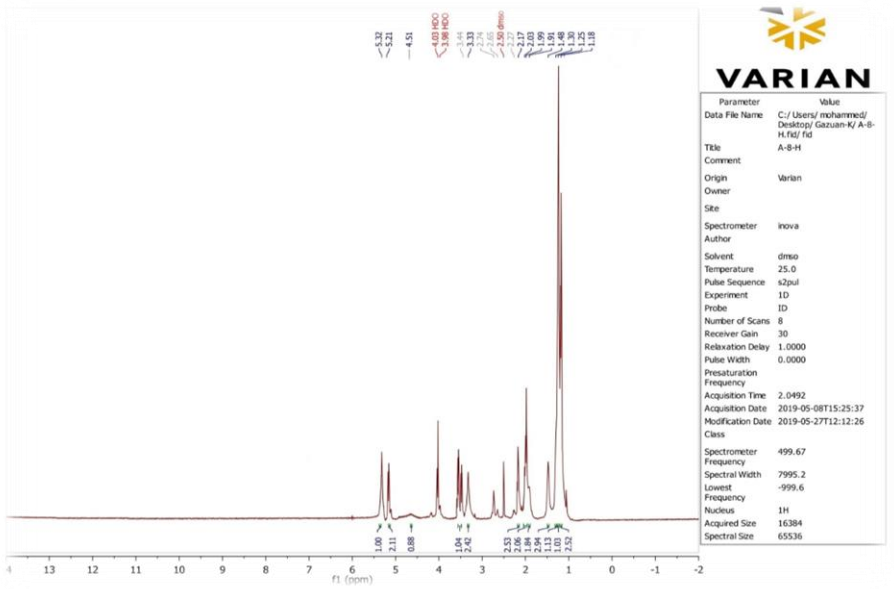


Fig. 1. ^{1}H.nmr spectrum

novel cancer treatment the traditional herbal drugs can be considered as minimum side effect treatment method (Fidelis, et al. 2019, Lauro, et al. 2019). The herbal drugs will play a much more animated role in the decreasing and cancer prohibition (Saad, Azaizeh, Said 2008). *Capris Spinoza* is one of the most prevalent medical plants, contained a wide range of phytochemical constituents, used extensively in several parts of the world as a treatment for many human diseases. Several records proved it is able to prevent various types of cancer cells (Al-Snafi, Yaseen & Al-Shatry 2015).

MATERIALS AND METHODS

Plant material

Capparis spinosa was collected from Kirkuk city during the month of July 2018. The plant identified by a botanist at Al Kirkuk University. The plant dried at room temperature in the dark for 14 days and then finely ground by using an electric grinder.

Extraction process

Capparis spinosa Seeds (20 g) and 150 ml of ethanol are introduced into a flask. The mixture was kept away from light and was put in ultrasound device under 45k for one hour for ten times this procedure under same condition has been done. Then the filtration process has been done on the mixture and the last volume (accumulate filtrate) is condensed in rotary evaporator under lower pressure.

Purification of the compounds using Silica Gel Chromatography

The Acetone extracted (1.00 g) was fractionated on a silica gel column (60-120 mesh) with ratio of eluent (Acetone: hexane) various such as (1:2) To give two fractions (Fr. 1- Fr. 2). part (2) was exposed to more chromatography on a silica gel column (1 m) with the usage of the identical eluate and using a step by step gradient of hexane and ethanol (2:1) to provide an another one pure compound (Fr.2.1) and mixture of others (13,14). The gained fraction I and II gives (150 mg and 140 mg respectively). Study of the effect of anabolic extract in acetone solvent on the effectiveness of cancer cells.

Validation and Detection of Cell Viability Using MTT Assay

The validation of MTT assay was performed to establish the relationship between cell number and absorbance. This is basically required to determine the accuracy of pipetting technique before moving to chemo-sensitivity assays. The MTT assay was conducted in accordance to Mossman (1983) with slight modulation. The cells were seeded in 45- well plates at a concentration of 0.7 x 105 cells/well. The cells were then incubated in a 37 °C CO2 incubator overnight. The next day, the synthesized chalcones compounds were added to the holes with 7 different densities. The cell viability was calculated at seventy-two hours post-treatment. MTT solution (4 mg/mL) was added at a volume of 20 mL in each hole and was incubated for 3 hours. Later, the solution was outlasted, and 100 mL of DMSO was added to all holes for the crystals solubilizing. Finally, the plates were read at 570 nm as the reference wavelength using µ-Quant ELISA Reader (Bio-tech Instruments, USA). The results of the study were statistically analysed using complete randomized design Using SPSS.

RESULT AND DISCUSSION

The (Al-Snafi, 2015). H.nmr spectrum of compound is shown in **Fig. 1**.

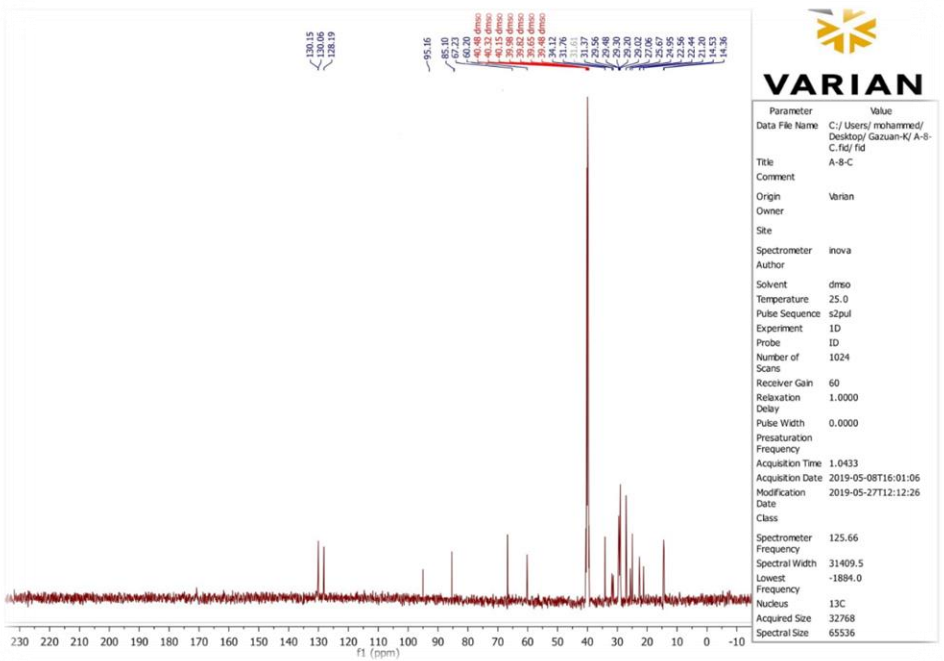


Fig. 2. C.nmr spectrum

Table 1. IR spectrum of compound

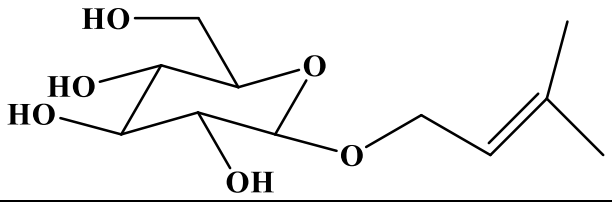
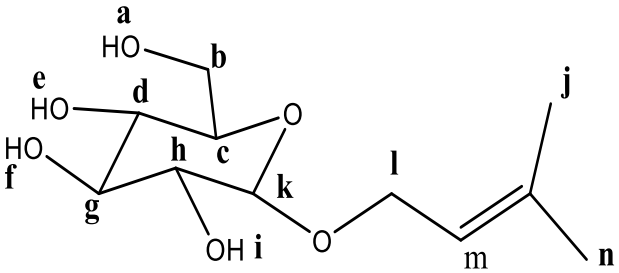
Compound	IR v cm^{-1} (KBr)
	3271 cm^{-1} of OH. 2889asym and 2835 sym of CH_2. 1595 cm-1 of C=C.

Table 2. Proton nuclear magnetic resonance spectrum of compound

Compound	^{1}H-nmr Chemical shift (ppm)
	j=1.48,3H,singlet. n=2.03,3H,singlet. h=2.17,1H,multiple. g and d=2.27,1H,doublet. f and e =3.33,1H,singlet. l=4.51,1H,singlet. l =5.21,2H, doblet. m =5.32, 1H, singlet. c and b =3.44,1H and 2H,multiple .

The (Saad, Azaizeh, Said 2008). C.nmr spectrum of compound is shown in **Fig. 2**.

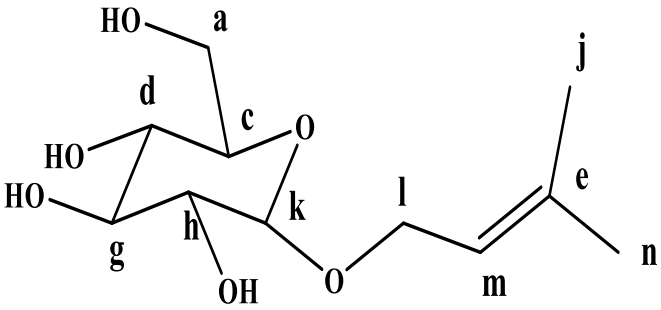
Detection of Cell Viability Using MTT Assay

Measure the effect of the compound extracted on the effectiveness of cancer cells by enzyme If **Table 3**, The adjusted table shows the standard deviation and standard error of the control A rate And the level of morale and the amount of confidence (higher and lower value and T.test.

Table 1, **Table 2** and **Table 3** shows spectrum of compound extract and **Table 3** shows a significant decrease in the efficacy of the enzyme coenzyme colin esters and at the level of probability (0.454 ± 0.015) compared to the control group where this decrease is a clear evidence of a compound effect obtained by killing cancer cells. We observe from the statistical analysis a significant decrease at the level of P≤0.05.and then estimated spectrum (IR,^{1}H.nmr and ^{13}Cnmr).

CONCLUSIONS

Table 3. Carbon nuclear magnetic resonance spectrum of compound

Compound	^{13}C-nmr Chemical shift (ppm)
	j= 14.53 n= 22.44 h= 24.59 a= 34.12 l= 60.20 d and g= 67.23 c= 85.10 k= 95.16 m= 128.19 e= 130.15

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