Study the Effect. Extracts and Inhibitory Potency of Plant *Capparis Spinosa* on Breast Cancer Cells

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Abstract

The aim of this study is to analyze Capparis spinosa and the principal constituent substance in Iraq which is recognized by NMR experimentations and the key constituent identified by NMR is Stachydrine. Capparis spinosa is descended from the family (Capparidaceae) and is described as a herbaceous plant with simple leaves together edge and forked ears and bear flowers on long necks and fruits pear shape. Capparis contains bitter materials, claycosides, enzymes, mayonnaise, acetic acid, bric, soap and volatile oils with a smell similar to the smell of garlic, as well as sulfuric klycosides. All parts of plants used as a treatment as roots that are used as a diuretic and leg used as a dentin for diarrhea as well as fruit is used as a treatment for cancer We observe measurements of significant decrease in the level of breast cancer cells at≥ 0.05

***Keywords*:** *Fruits of Capparis spinosa:; Ethanol;Aceton and Hexane.*

Introduction

Capparis spinosa belongs to the family family (Capparidaceae) and is described as a herbaceous plant with simple leaves together edge and forked ears and bear flowerson long necks and fruits pear shape [6]. Shafahalah contains bitter materials, claycosides, enzymes, mayonnaise, acetic acid, bric, soap and volatile oils with a smell similar to the smell of garlic, as well as sulfuric klycosides [8]. All parts of plants used as a treatment as roots that are used as a diuretic and leg used as a dentin for diarrhea as well as fruit is used as a treatment for cancer [6]. Common names in Arabic: Kabar, Asef; Berbe: Tayllut, Tailoulout, Amserlih, Ouailoulou; English: Caper bush, Caperbush, Caper, Caperberry; French: Câprier, Capriercommun, Câpres, Fabagelle, Tapana, Finnish: Kapris; German: Kapper, Kapernstrauch ; Gujarati: Kabare; Hindi: Kiari, Kobra; Hungaria: Kapricserje; Icelandic: Kapers; Italian: Cappero, Capperone (fruit); Kannada: Mullukattari; Maltese: Kappara; Marathi :Kabar; Norwegian: Kapers; Portuguese: Alcaparra; Punjabi: Kabarra; Russian: Kapersy; Sanskrit: Ahimsra, Kanthari, Kantaka, Tiksnagandha; Spanish: Alcaparra, Caparra, Tapana; Alcaparron, Caperberries; Swedish: Kapris; Telugu: Kokilakshmu; Urdu: Kabar [1-3]. Family : Capparidaceae. Prepar Distribution It is supposed to be originated in the dehydrated places of Western and Central Asia. Currently, Capers is existed maturing all over Mediterranean (especially in France, Spain, Italy and Algeria); furthermore, the plant is found in Iran, Iraq, Cyprus and Greece [3-4]. Globally, human population is highly and severely affected by a disease known as cancer. A steadfast requirement for fresh remedies to cure, treat and curb off this life- menacing disease. Many key strategies such as controlling survival and the death of a cell which are cancerous are essential for managing and treating cancer. With lesser possible side effect on normal cells, anticancer agents should kill the cancerous cell. Recently, medicinal plants having natural products to deter proliferation, prompt apoptosis, curb off angiogenesis, constrain invesiveness, in addition to retarding metastasis and improving chemotherapy [1-7]. The present review discusses the compounds derived from natural medicinal plants and their assets which lead to maintaining them promising possible treatments for anticancer.

Anti--cancer Activity of Medicinal Plants

Various advantages for Medicinal plants are

overcoming chemical products, since compounds derived from plants are more allowed and treated as untoxic to the normal cells of human. The current common therapeutic remedies for treating cancer have already been used by radiotherapy and chemotherapy having different side effects such as toxicity in neurology, heart effects, kidney and lung which seriously damages the person‘s health. Consequently, the less toxic compelling anti-cancer medication as compare to the remedies in the marketplace are considered as a required alternative method to develop the drug.

Capparis spinosa

The Capparaceaen family include Capparis spinosa which is treated as key culinary components in theMediterranean and Middle Eastern cuisines. This component is Sanskritlly recognized as HimsraCabra. Generally, Caper forms different constituents such as flavonol glycoside, rutinand 5-caffeoyl-quinic acid which can be volatile and nonvolatile which could also be effective anticancer agents. Susequently, a protein similar to imidazoleglycerol phosphate synthase is definitly refined from garden-fresh Caper seeds, deterring the process of proliferating hepatoma HepG2 cells, in addition to the colon cancer HT29 cells as well as MCF-7 cells in breast cancer [13]. Principal oils and aqueous infusions taken from Caper have revealed substantial inhibitory impact on HT-29 cell proliferation and on nuclear factor kB (NF-kB) action in a drug prescription dependent mode. The cells in G2/ M phase of cell cycle were ceased by Caper essential oil and aqueous infusion. A study showed that C. S obtain mediated apoptosis through permeabliztion of mitochondria in addition to acting of Caspase 9 in SGC-7901 cells [14].

The other key cause of death universally is cancer. Procedures of treatment are more complicated for each detailed type of cancer aiming for anti-cancer drugs with higher effectiveness.

1. The pharmaceutical societies many advancements achieved in cancer treatment strategies, less toxicities and lower expenses. In spite of reporting drug-resistance in treating several cancer tryouts, it is illustrated by the second studies on the experiment referring that some wellknown plants possess anti-cancer outcomes on different tissue cells, for the various compounds included in plant drugs, on the contrary to the virtuously synthesized story. IIt sounds that showing novel kinds of drugs introduced and extracted from plants could pave the way to more competent approaches to treating cancer. Over years, considerable attention has recently been paid, mostly due to vast developments3 to drugs derived from plants in organic chemistry, and due to groundbreaking changes in the methods used in extracting and purifying more accurate understanding of the plants' natural ingredients. Making use of plant sources as raw material may be assistant to the pharmaceutical industries to develop enhanced drugs with cheaper expenses. Capparidaceae is a great family of phanerogam gymnosperm dialypetalae plant species. The Capparis spinosa family, not only shows noticeable resistance to family, not only shows noticeable resistance. The Capparispinosa (C. spinosa) (CS) as an individual memeber of the family of Capparidacae, not just maintaining apparent endurance to shortage in water supplies and increasing temperatures, but adapting decreased temperatures with -8 C 5. Flavonoids are recognized as being the biggest group of natural constituents that are considered as strong anti--oxidants and having noticeable impacts in cellular biology, e.g. accumulating free radicals and possibly halting their detrimental role in carcino-genesis 6. Like all other betaines, Stachydrine is a compound of quaternary ammonium. In the vegetal realm, betaines are omnipresent which tending to gather and assemble in the intercellular fluids and cytoplasm in which they perform an instrumental role in keeping up proteins, nucleic acids, and cell membranes against abiotic stress (Kavi Kishor et al., 2005; Street, Bolen, Rose, 2006). In a particular study, cells with high-glucose (30 mM) culturlly modelled was employed to simulating clinical hyperglycemia for the invitro estimation of the influence of stachydrine on high-glucose induced cytotoxicity. It is clearly recognized that stachydrine, which is an full of ingredient of citrus juices, is capable of preventing the high-glucose cytotoxicity in endothelial cell by working on the senescence and S I R T 1 pathways (Servillo et al. , 2013).

Anticancer Activity

Cancer, was described as, cell proliferation affecting other tissues or an uncontrolled growth. The mechanism beyond invading the tissue is by direct cell migration or lymphatic system and blood. The hazard agents for

cancer includes an unhealthy diet, environmental factors, infection, radiations, chemicals and smoking [17]. 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 stage» or malignant «end stage, called cancer» . Natural products from biological sources and plants still stay an uncondensed source and unlimited of new nutraceuticals and phytochemicals. Itcontinues to produce the most dramatic effect in the field of cancer [18]. Traditional herbal founded drugs could be hopeful candidates for novel cancer treatments with minimal side effects. They play a significant and vital role in the reduction and prevention of cancer (Saad et al., 2008). Capparisspinosa is one of the mostprevalent medicinal plants, contained a wide range of phytochemical constituents, used extensively in several parts of the world as a treatment for many human diseases. Many reports confirmed it isable to inhibit many types of cancer cells [5].

A new protein is separated from caper, which inhibited proliferation of MCF-7 (breast cancer), HepG2 (hepatoma), and HT29 (colon cancer) cells [6]. Capparis spinosa essential oil and aqueous infusion appeared marked a prohibitory effect on nuclear factor kappa B (NF-KB) activity and on HT-29 cell proliferation [23]. Furthermore, C. Spinoza extracts mediated apoptosis through the mitochondrial pathway in SGC-7901 cells (Ji and Yu 2014).The aqueous leaf extracts of caper induced significant inhibitory effect on Hep-2 «human epidermoid larynx carcinoma» and Hela «human cervix uteri epitheloid carcinoma» with low concentration (Al-Daraji 2010).Also, different extracts (aqueous and methanolic) of C. spinosa fruit showed Cytotoxic and cytogenetics effects on in vitro Hep-2 and HeLa tumor cell lines (AL-Asady et al., 2012). Finally, the extract of C. spinosaroot bark revealed anticancer volume of tumor and packed cellsbesides cell count viability [26]

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 of 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 (10 g) and 100 ml of ethanol are introduced into a flask. The mixture was exposed to ultrasound for 1 hour under 45 k at temperature and sheltered from light. The later process has been repeated under same condition for ten times. Afterward, the mixture was filtrated and the final volume (accumulate filtrate) is concentrated in rotary evaporator under reduced pressure (12).

Purification of the compounds using Silica Gel Chromatography

The Acetone extracted (2.00g) was absolutly fractionated on a silieca - gel column (60-120 mesh) with ratio of eluent (methanol ;Acetone,hexane) various such as (1;2:3) To give two fractions (Fr. 1- Fr. 2). Fraction (2) was dependent on further chromatography on a silieca - gel column (1 m) with same eluate using a stepwise gradient of hexane and ethanol (2:1) to withstand further pure compound (Fr.2.1) and mixture of others [13,14]. The obtained fraction I and II gives (170 mg and 143 mg respectively).

Study of the effect of anabolic extract in acetone solvent on the effectiveness of cancer cells.

Result and Discussion

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 analysis was carried out in conformity with Mossman (1983) with some adjustments. The cells were planted in 45- well plates at 0.8 x 105 cells/well concentration, incubating the cell in a 37 °C CO2 incubator during the night. The next day, the chalcones compounds which were synthesized were added to the wells with 7 various concentrations. Measuring the viability of the cell at 72 hours after treatment. A volume of 20 mL MTT solution (5 mg/mL) was added in each well and incubated cell for three hours. Then discarding the solution where 100 mL of DMSO was supplementary to each well for solubilizing the crystals. Lastly, the plates were rated at 570 nm as

reference wavelength by using µ-Quant ELISA Reader (Bio-tech Instruments, USA). (Nadiah et al., 2014) The results of the study were statistically analysed using complete randomized design Using SPSS.

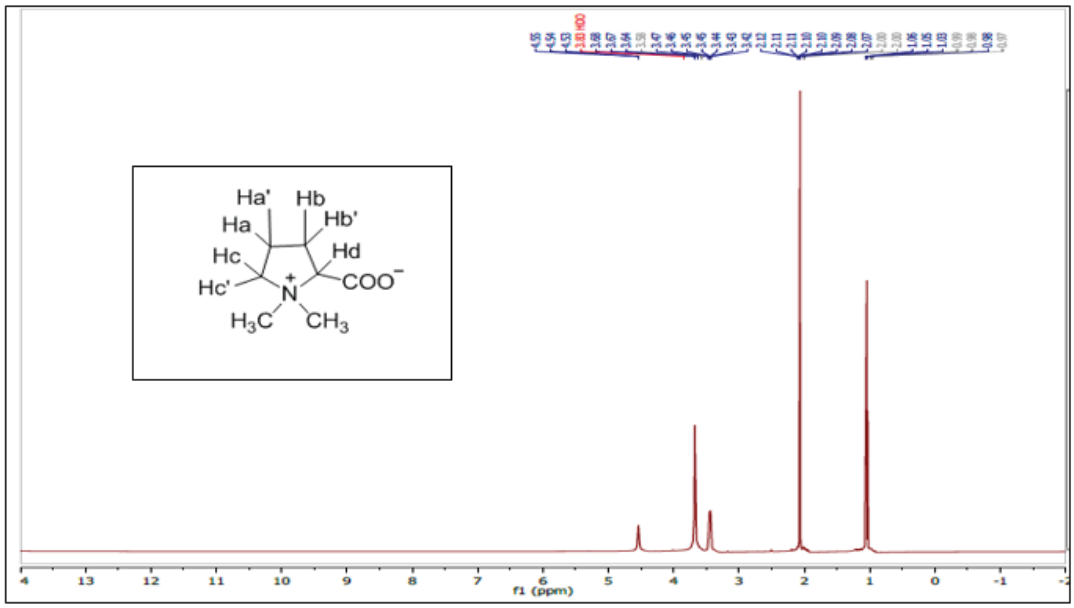

Figure 1: design Using SPSS

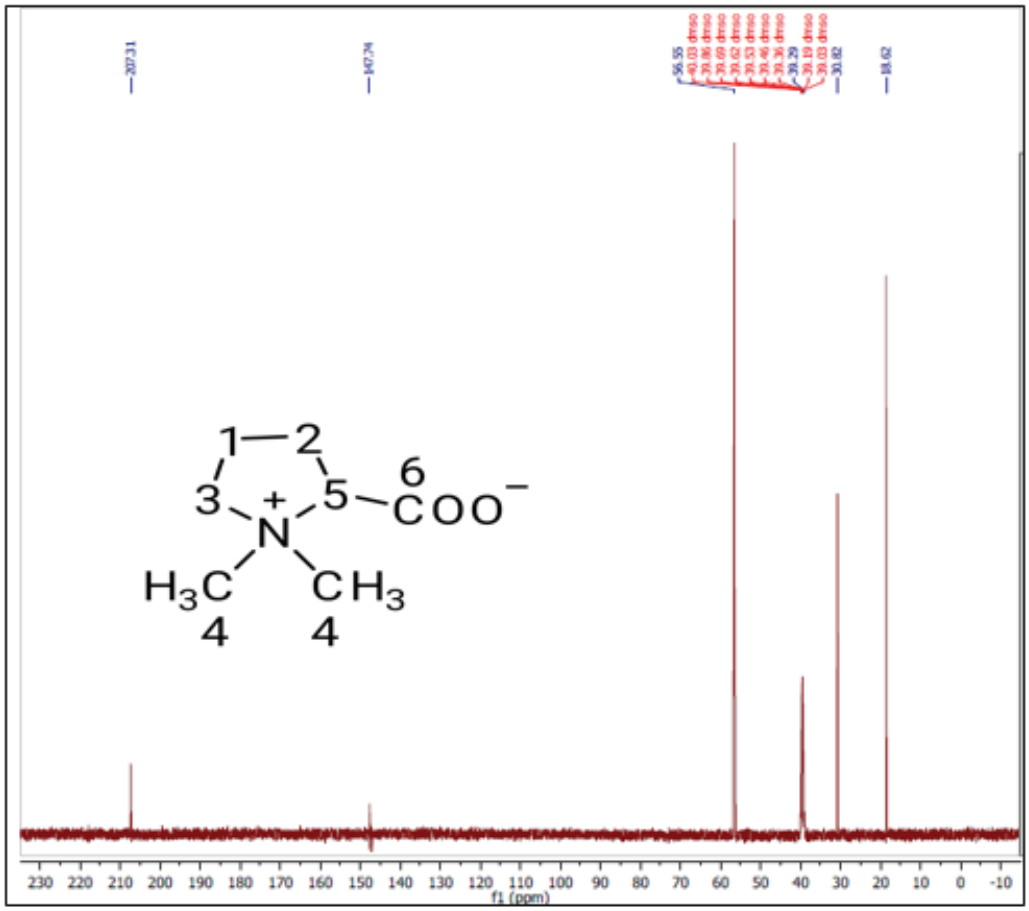

Figure 2: ^{13}C NMR

Figure 2: (a) The 1H NMR spectrum of (Stachydrine compound) is noticed in fig. 1. The spectrum shows one characteristic singlet peak at 2.26 ppm due to the protons in (CH3)2-N. A pentate centered at 1.30 ppm is assigned to the protons (a, a'). A multiplete at 1.70 to 1.95 ppm is attributed to the protons in (b, b'), peaks at 2.20 to 2.30 ppm is assigned to the protons in (c, c'). Finally, triplet peak appeared at 3.12 ppm is attributed to the proton in (d). Furthermore, the conformation is carried out in 13C NMR compound (I) shows six different peaks are ranged from 24.7 to 174.7 ppm (C1 at 24.7 ppm, C2 at 28.8 ppm, C3 at 60.1 ppm, C4 at 41.4 ppm, C5 at 76.3 ppm and C6 174.7 ppm). Both NMR is conformed the chemical structure of compound (I).

Detection of Cell Viability Using MTT Assay.

Measure the effect of the compound extracted on the effectiveness of cancer cells by enzyme If the table (3-1), 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: the standard deviation and standard error of the control A rate

Group	N	mean±SD	t.test	p-value
Control	25	1.23±0.05	0.02	0.054
Sample	30	0.554±0.015		

Conclusions

The result shows a significant decrease in the efficacy of the enzyme coenzyme colin esters and at the level of probability (0.554 ± 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.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Education and all experiments were carried out in accordance with approved guidelines.

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