

CYTOTOXIC EFFECTS OF JORDANIAN SIMMONDSIA CHINENSIS (LINK) C.K. SCHNEID ON DIFFERENT CANCER CELL LINES

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Abstract

Context: Different parts of *Jojoba* (*Simmondsia chinensis* (Link) C.K. Schneid) from the family *Simmondsiaceae* have been traditionally used in folklore-medicine for skin disorders, wound healing, tumors and many other uses.

Objective: The aim was to examine the effect of the total extracts of *Jojoba* leaves (male & female), testa, seeds and oil on the growth of various cancer cell lines.

Materials and methods: Plant parts were subjected for hot extraction by solvents of different polarity (hexane, methanol and ethanol), in addition to cold pressing extraction of seed. Furthermore, the active compounds were isolated by preparative thin layer chromatography, followed by identification with chemical reagents along with various methods of chromatography and spectral analysis for simmondsin and three of its derivatives namely Simmondsin-3'-ferulate, 4,5-Didemethylsimmondsin and 4-Demethylsimmondsin -2'- ferulate. In addition, phenolic compounds, phytosterols, tocopherols and fatty acids were also identified by the same methods. The evaluation of the acute cytotoxic effect of the extracts on human cancer cell lines using human melanoma (MV 3), breast (MCF 7),

and colorectal (HCT 116) tumor cell lines, was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Results: According the IC₅₀ values, both *jojoba* oil (hexane extract & cold pressing extract) and methanolic extract of testa have antitumor activity, more potent than the other parts, against HCT 116, MV 3 cancer cells. Moreover, *Jojoba* extracts induced a dose-dependent cytotoxicity.

Discussion and conclusion: Plant extracts induced variable degrees of cytotoxicity against cancer cell lines due to their active constituents which were observed in different parts of the plant.

Keywords: Testa, simmondsin, simmondsin ferulate, *jojoba* oil, MV 3, MCF 7, HCT 116, MTT

Introduction

Cancer is a leading cause of mortality, which strikes more than one-third of the world's population. It is also responsible for more than 20% of all death cases (Zaid et al., 2012). Besides it has been determined that over 60% of the anticancer drugs in current use are in some way derived from natural sources (Heinrich et al., 2008).

Moreover, many glycosides have been found to have anticancer and tumor preventive properties (James et al., 1996). Also, the lipids have a role in cancer as observed in the signaling for the phagocytosis of apoptotic cells (Elliott & Ravichandran, 2010). In addition, Vitamin E might also block the formation of carcinogenic nitrosamines formed in the stomach from nitrites in foods and protect against cancer by enhancing immune function (Weitberg, 1997). Furthermore, the studies showed that phenolic compounds have antimutagenic (Lazarou et al., 2007), anticarcinogenic (Kuroda, 1999) and antiproliferative actions (Matito et al., 2003). Also, tannins showed anti-tumor activity while ellagic acid had anti-mutagenic effects (Bhat et al., 2005).

Simmondsia chinensis (Link) C.K. Schneid is the only member in its family (*Simmondsiaceae*) and it known's as *Jojoba*, which was originally derived from the Sonoran, Baja and Mojave deserts of Arizona, California in the USA as well as in Mexico. It is presently distributed over many countries. In Jordan, it is cultivated in the Jordan University of Science & Technology farms since 1986 and recently in Al-Ghoor area (Al-Zoubi, 1996). *S. chinensis* is unique in many ways (Gentry, 1958). It was the Native American Indians who discovered the importance and versatility of *jojoba* especially the folklore-medicine use as remedy for different tumors (De beer, 2009; Orwa et al., 2009).

Jojoba seeds produce 45% to 75% by weight of a colorless, odorless oil or liquid wax. This oil has a unique chemical structure in the plant world

(Elias-Cesnik, 1982). The wax is primarily-97%- composed of straight chain acids and alcohols with two double bonds. In addition, it contains sterols and stanols (Tada et al., 2005) along with trace amounts of triglyceride (Van Boven et al., 2000 c) and different toccopherols mainly α -toccopherol (El-Mallah, 2009). Furthermore, groups of nitrile glycosides known as simmondsin's compounds (10% to 20%) have been identified (Van Boven et al., 2000 a; Laszlo et al., 2006). These simmondsins are considered unusual nitrile glycosides and cannot be compared to most other nitrile glycosides found in plants (Allen, 1997). Besides *Jojoba* seeds and meal have been shown to contain considerable amounts of tannins (2.5%) (Wiseman, 1987 a, b). In addition, *Jojoba* contains anthocyanins namely malvidin (Sharp, 1974), alkaloids (Hultin, 1966), while the leaves contain two major flavonoids which are isorhamnetin 3- rutinoside (narcissin) and isorhamnetin 3, 7-dirhamnoside (Orwa et al., 2009).

Materials and methods

The plant materials of *S. chinensis* were cultivated in the farms of Jordan University of Science and Technology (JUST) / Irbid-Jordan, and were collected during the months of October, November and December (2011), with the co-operation of Agricultural Services Department in JUST. Plant specimens were identified with the help of the plant taxonomist, and checked with a herbarium collection at the department of biological sciences, Faculty of Science in the University of Jordan. Each specimen classification was verified by Professor Dawud Mohammad Hasan Al-Eisawi in the Faculty of Science, Department of Biological Sciences.

The leaves (male & female), seeds and testa were dried at room temperature (25°C) in the shade for about 15 days and then milled and weighed. Cell Lines; Human melanoma (MV 3), breast (MCF 7), and colorectal (HCT 116) cell lines were generously provided by Dr. Rick F. Thorne (University of Newcastle, Australia) and were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% Fetal bovine serum (FBS) (Bio Whittaker, Verviers, Belgium). The 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was purchased from Molecular Probes (Eugene, UR).

Extraction methods

The extraction of simmondsin and other related compounds was carried out using the reported methods of (Holser, 1999) and (Zaher et al., 2004). Different parts of *S. chinensis* were used namely: seed, testa, male and female leaves. The samples were soaked in *n*-hexane (95%) followed by hot extraction using soxhlet apparatus for 12 hours. The contents were filtered and dried. Different solvent were added separately (ethanol and methanol) in

different patches and refluxed for 12 hours, with optimum control of temperature and pH. The extract was evaporated under reduced pressure using rotatory evaporator. The liquid wax extracts were kept in amber glass containers to avoid any photo-oxidation.

Another extraction method was used for obtaining liquid wax extract and other related compounds, after complete removal of testa from the seeds. This method is based on cold pressing technique by hydraulic presser machine designed specifically for this purpose in the *Jojoba's* cold-pressing unit, a part from the Agricultural Services Department in JUST (AI-Zoubi, 1996). All different extracts were dried, cooled and subjected to identification process. General scheme for extraction methods is shown in Figure (1).

Screening for the presences of the active constituents

Screening for the presence of the active constituents {nitrile glycosides, phenolic compounds, tannins, fixed oil (fatty acids), phytosterols and toccopherols} is performed by analytical thin layer chromatography technique. This was performed using specific mobile phases for each group of compounds: ethyl acetate/ethanol (70:30, v/v) (Wagdy et al., 2011), chloroform/methanol (80:20, v/v) (Van Boven et al., 2000 b) and benzene (100, v/v) (Spencer & List, 1988). This was followed by detection using ultra violet light and certain chemical reagents.

The identification reagents for nitrile glycosides were: (a) 1-naphthol spray (Van Boven et al., 2001), (b) sulfuric acid (10%) spray (Van Boven et al., 2000 b), and (c) sodium picrate solution (Müller-Schwarze, 2009). The identification reagents for phenolic compounds and tannins were: (a) 3-5 % w/v solution of ferric chloride in ethanol (95%), and (b) sulfuric acid (10%) spray (Van Boven et al., 2000 b). The identification reagents for fixed oil (fatty acids), phytosterols and toccopherols were: (a) iodine vapor (Tada et al., 2005), and (b) a mixture of concentrated sulfuric acid in ethanol (50:50, v/v) spray and heating the TLC plates in a 100°C oven for 15 min (Van Boven et al., 2000 c).

Isolation of the active constituents

Isolation was carried out by using preparative TLC plates of glass support, silica gel matrix, with fluorescent indicator λ_{\max} 254 nm and λ_{\max} 365 nm, 20 × 20 cm, thickness 1 mm (Machery-Nagel, Germany). The mobile phase used was chloroform/methanol (80:20, v/v) for identification of nitrile glycosides, phenolic compounds and tannins. Many separated bands were observed under UV light of λ_{\max} 254 nm and λ_{\max} 365 nm. These bands showed a clear color response to the chemical reagents also giving Retardation factor (R_f) values similar to those observed by the chemical

standards used. The standards were: simmondsin, simmondsin-3'-ferulate, 4, 5-didemethylsimmondsin, 4-demethylsimmondsin-2'- ferulate, caffeic acid, gallic acid, tannic acid and ferulic acid.

Cell viability assay

The acute cytotoxic effect of the extracts on human cancer cells was determined using MTT assays. Cells were seeded at 5000/well onto flat-bottomed 96-well culture plates and allowed to grow for 24 hours before the desired treatment. Cells were incubated with a wide range of the extract concentrations (0-200 µg/ml) for 72 hours. Then cell growth was evaluated by labeling the cells with MTT from the Vybrant MTT cell proliferation assay kit (Molecular Probes, Eugene-UR). The resulting formazan was solubilized with dimethyl sulfoxide (DMSO). Absorbance was read in a microplate reader at λ_{max} 540 nm. The percentage cell viability was calculated with the appropriate controls taken into account. The concentration which inhibited 50% of cellular growth (IC₅₀ value) was determined. Cytotoxicity of the sample towards the cancer cells was expressed as IC₅₀ values. The inhibitory rate of cell proliferation was calculated by the following formula:

$$\text{Growth inhibition (\%)} = \frac{\text{absorbance control} - \text{absorbance treated}}{\text{absorbance control}} \times 100$$

Statistics

Analysis was performed using the Microsoft Office Excel (2009, Microsoft-USA) for the calculations of the (IC₅₀) of the extracts. Statistical Product and Service Solutions (SPSS) Statistics version 17.0 (2008) / International Business Machines Corporation (IBM)-USA software was used to perform the analysis of variance (ANOVA) tests, which were used to determine if there were any statistically significant differences in the inhibition effect of the extracts on the tumor cell lines. *P*-values < 0.0167 were considered significant.

Results

Cell viability assay

To examine the antitumor potential of *Jojoba*, cell viability analysis was performed. As shown in Table (1), *Jojoba* extracts induced a dose-dependent cytotoxicity. Human colorectal (HCT 116) cancer cells were highly sensitive to *Jojoba* seed oil extracted by either hexane or cold pressing. Moreover these cells were intermediately sensitive to testa methanolic extract, supernatant from seed ethanolic extract and (male & female) leaves ethanolic extracts. Furthermore these cells were weakly sensitive to seed ethanolic extract. In addition, human melanoma (MV 3)

skin cancer cells were highly sensitive to *Jojoba* seed oil extracted by hexane. Also, these cells were intermediately sensitive to testa methanolic extract, (male & female) leaves ethanolic extracts, seed ethanolic extract and supernatant from seed ethanolic extract.

Furthermore, these cells were weakly sensitive to *Jojoba* seed oil extracted by cold pressing. In addition, human breast (MCF 7) cancer cells were intermediately sensitive to male leaves ethanolic extracts, seed ethanolic extract as well as *Jojoba* seed oil extracted by cold pressing. These cells were weakly sensitive to *Jojoba* seed oil extracted by hexane, supernatant from seed ethanolic extract, female leaves ethanolic extracts and testa methanolic extract. The relation between growth inhibition and *Jojoba* extracts' concentrations according to the type of cell line is shown in Figure (2). In addition, the relation between growth inhibition and *Jojoba* extracts' concentrations according the *Jojoba* extracts' types is illustrated in Figure (3). Furthermore, the relation between cell lines, *Jojoba* extracts' types and growth inhibition is shown in Figure (4).

Statistical analysis

Three two-way analyses of variance (ANOVA) were conducted to investigate the IC_{50} differences on the factors of plant parts, concentration of the extract and types of tumor cell line. Therefore, the three two-way ANOVAs included investigating the IC_{50} differences on: (a) concentration of the extract and type of tumor cell line (concentration X cell line), (b) plant parts and type of tumor cell line (part X cell line), and (c) plant parts and concentration of the extract (part X concentration). Prior to examining the ANOVA results, the alpha level was adjusted utilizing the Bonferroni adjustment. Accordingly, the adjusted α -level was determined to be 0.0167.

The concentration X cell line univariate two-way ANOVA results, reveal no significant two-way interaction between concentration of the extract and type of tumor cell line on the dependent variable of IC_{50} . Consequently, the main effects for concentration and cell line were examined and revealed a significant main effect for concentration but not cell line. The part X cell line results, indicate that there is no significant two-way interaction between plant parts and type of tumor cell line on the dependent variable of IC_{50} . The concentration X part results showed no significant two-way interaction between concentration of the extract and plant part on the dependent variable of IC_{50} . Consequently, the main effects for concentration and part were examined and revealed a significant main effect for concentration but not part.

Discussion

These results, which were obtained after studying three different tumor cell lines came from three different types of cancers, preliminary clarify that Jordanian *jojoba* possess anticancer activity. According to the IC_{50} values, both *jojoba* oil (hexane extract & cold pressing extract) and testa (methanolic extract) have this activity. Based on literature review, there were no previous studies that explored the anticancer activity of *S. chinensis* of different plant parts extractions except (Elnimiri & Nimir, 2011) study. They investigated the anticancer activity for *jojoba* oil only on one tumor cell line and the results were negative. Secondary metabolites may have potential anticancer activity, either by direct cytotoxic effect on cancer cells or by modulating the course of tumor development, and eventually inhibit it (Kintzios et al., 2004).

The results of this study showed that the cytotoxic activity on the cancer cell lines increased as the concentration of the extracts increased. This is known as dose-dependent phenomena (Heslam, 1996). There was also a certain degree of selectivity of the extracts towards the cancer cell, because the cancer cell differs from the normal cell by its metabolic reactions and cell receptors (Geissman, 1963). These extracts showed cytotoxic activity against different cancer cell lines used. This activity may be due to the presence of caffeic acid, ferulic acid, gallic acid, tannic acid and group of phytosterols like sitosterol, campesterol, stigmasterol, avenasterol and brassicasterol in addition to saturated and unsaturated fatty acids, which are present in the extracts of *S. chinensis*.

Phenolic compounds could be cytotoxic against certain cancer types (Kumarappan, 2007; Ismail et al., 2012). Different types of phytosterols have been identified in nature. In recent years, a great attention has been given to the role of phytosterol in the protection from cancer. They act at various stages of tumor development, including inhibition of tumorigenesis, inhibition of tumor promotion, and induction of cell differentiation (Viana et al., 2004; Woyengo et al., 2009).

Furthermore, it has been shown that *Jojoba* contains a group of nitrile glycosides known as simmondsin's, these pharmacologically active substances act as anti-inflammatory (EI Mogy, 2003; Habashy et al., 2005; De beer, 2009) and angiogenesis inhibitors (D'Oosterlynck, 2001; D'Oosterlynck, 2008). These nitrile glycosides may have a role in the anticancer activity. The glycosides could be cytotoxic against certain types of cancer and also demonstrate antiviral and antileukemic properties (Kintzios et al., 2004).

Moreover, *Jojoba* oil contains fatty acids, and in recent years, a great deal of interest has been given to the role of fatty acids in the protection from cancer or affects tumor growth. Several studies suggested that omega-3 and

omega-6 are cytotoxic to different types of cancer cells and may act synergistically with current chemotherapeutic drugs (Comba et al. 2010).

Conclusion

Since *Jojoba* plant is cultivated successfully in Jordan, it is worth to mention that this is the first work in Jordan regarding biological evaluation.

Jojoba contains group of nitrile glycosides and they are not cyanogenic.

Crude extracts from different parts of *S. chinensis* exhibit antitumor activity on different types of human cancer cell line. According the IC₅₀ values, both *jojoba* oil (hexane extract & cold pressing extract) and methanolic extract of testa have this activity, more potent than the other parts, against human colorectal (HCT 116), human melanoma (MV 3) cancer cells. Moreover, *Jojoba* extracts induced a dose-dependent cytotoxicity.

This is a pioneer results regarding to *Jojoba* which is cultivated in Jordan under different environmental conditions.

Recommendations

Further work on the chemical constituents and biological activity of flowers (male & female) and roots of *S. chinensis*.

Detailed further investigation regarding the differences in the chemical constituents and biological activity between the male and female *Jojoba* leaves, since this study clarify that there were differences in chemical composition (by TLC & HPLC) between the two genders of leaves since this study clearly shows that *Jojoba* leaves possess both antioxidant and anticancer activities more potent than other *Jojoba* plant parts.

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Tables and Figures

Table 1. Anticancer activity of *Jojoba* extracts from different selected parts prepared in different methods.

<i>Jojoba</i> Extracts	Cell lines IC ₅₀ (µg/ml)		
	HCT 116	MV 3	MCF-7
Female leaves (ethanolic extract)	83.1	71	111.39
Male leaves (ethanolic extract)	87.99	65.86	81.49
Seeds (ethanolic extract)	107.45	82.01	89.43
Seeds' supernatant (ethanolic extract)	55.23	84.92	105.01
Testa (methanolic extract)	47.19	58.51	114.74
Seeds oil (hexane extract)	7.11	39.91	103.95
Seeds oil (cold pressing extract)	19.29	100	95.9

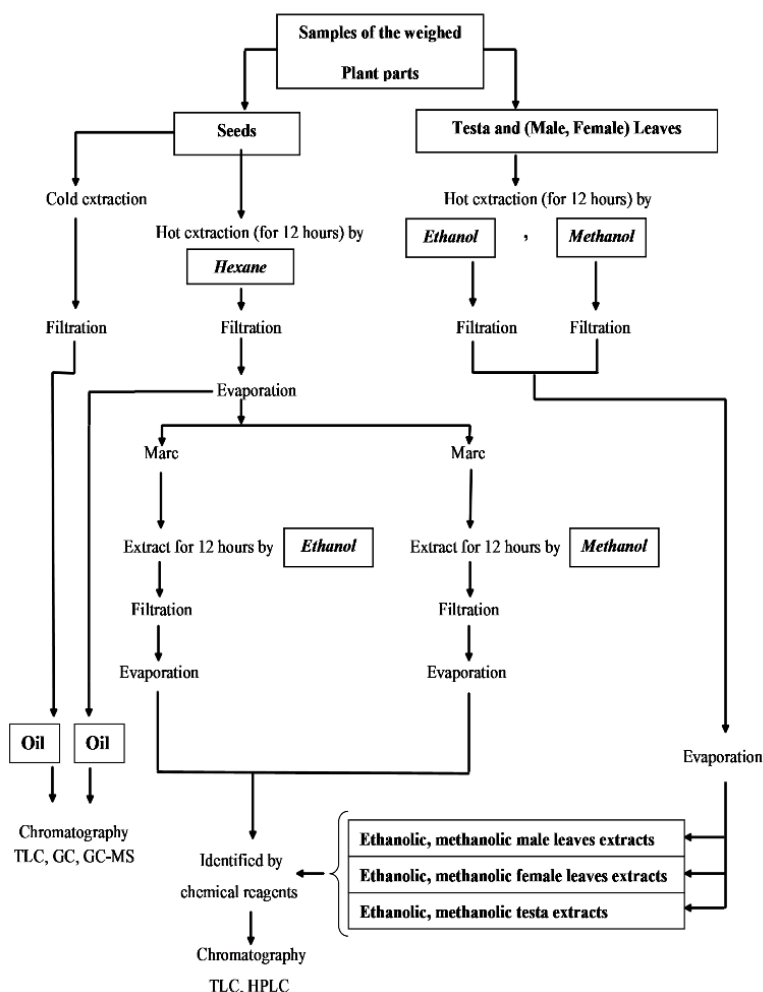


Figure 1. General scheme for extraction methods

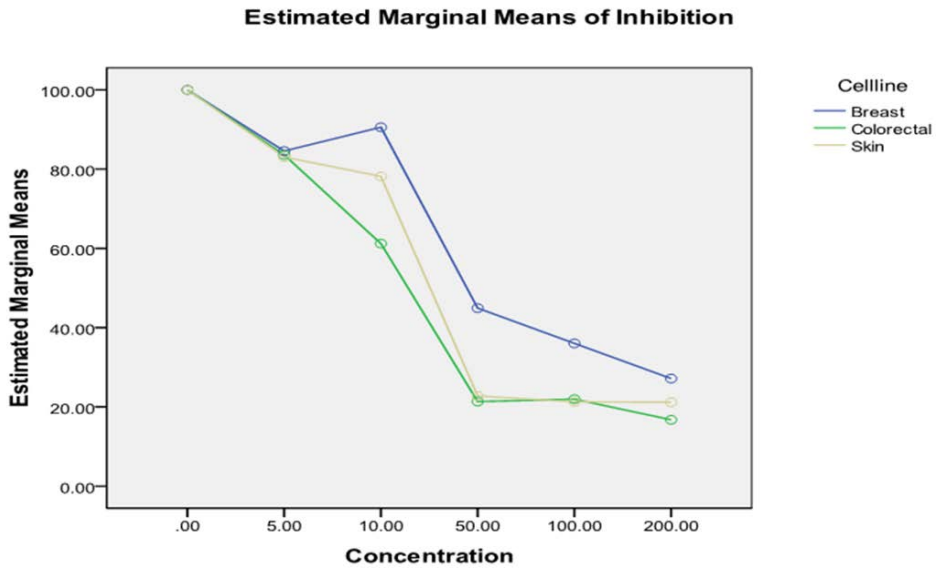
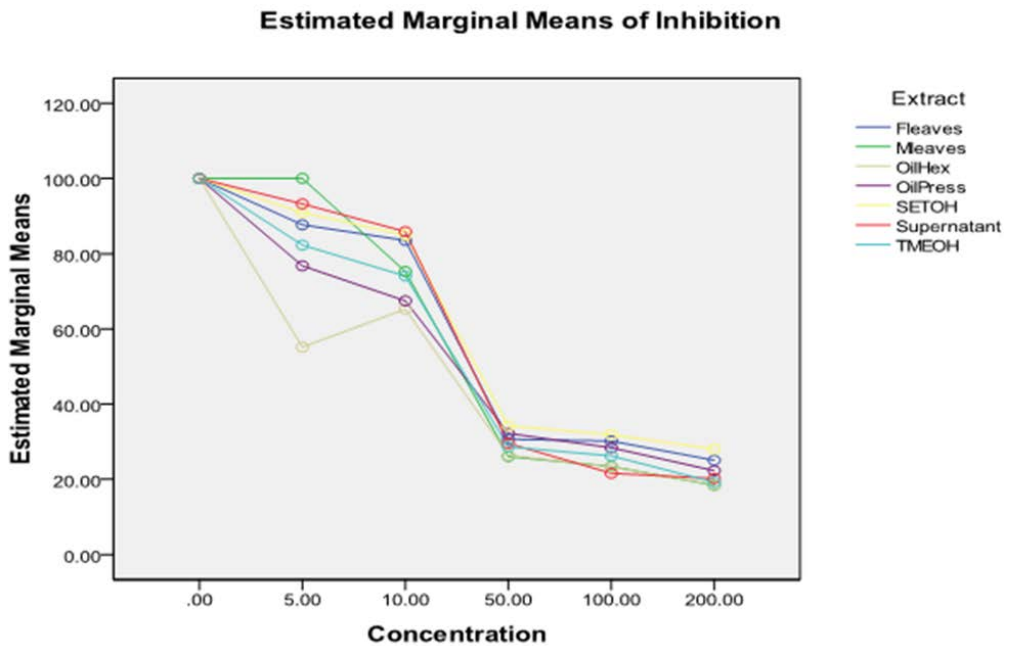
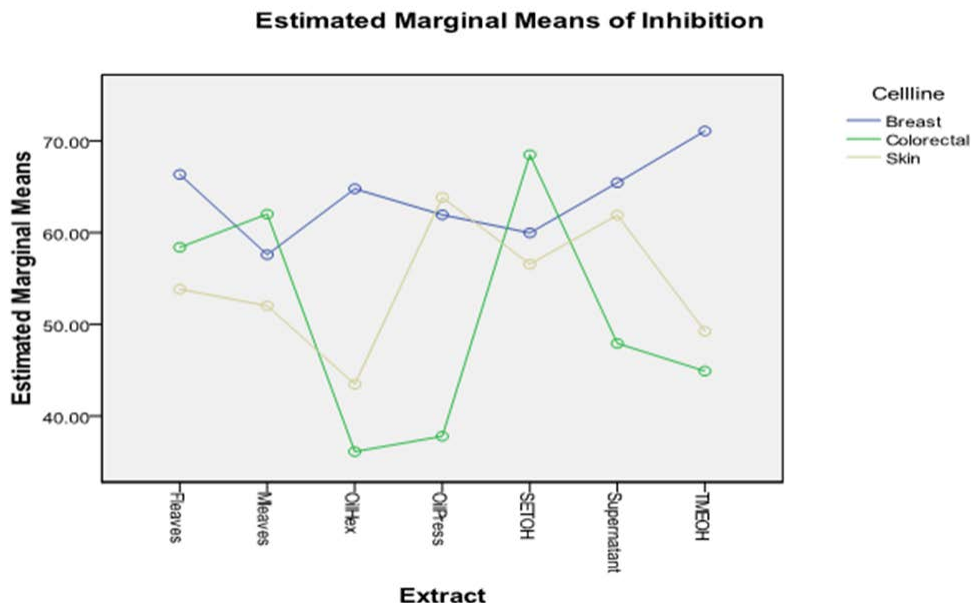


Figure 2. The relation between growth inhibition and Jojoba extracts' concentrations according the type of cell line



Fleaves=Female leaves (ethanolic extract), Mleaves=Male leaves (ethanolic extract),
 OilHex=Seeds oil (hexane extract), OilPress=Seeds oil (cold pressing extract),
 SETOH=Seeds (ethanolic extract), Supernatant=Seeds' supernatant (ethanolic extract),
 TMEOH=Testa (methanolic extract)

Figure 3. The relation between growth inhibition and Jojoba extracts' concentrations according the Jojoba extracts' type



Fleaves=Female leaves (ethanolic extract), Mleaves=Male leaves (ethanolic extract), OilHex=Seeds oil (hexane extract), OilPress=Seeds oil (cold pressing extract), SETOH=Seeds (ethanolic extract), Supernatant=Seeds’ supernatant (ethanolic extract), TMEOH=Testa (methanolic extract)

Figure 4. The relation between cell lines, Jojoba extracts’ types and growth inhibition

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