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The Fatty Acid Composition and the MTT-Cytotoxicity Test of Commercially Available *Commiphora Gileadensis* Balsam Oil

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Abstract:
Commiphora gileadensis, an aromatic plant, is traditionally used in Middle Eastern countries to prevent pain and inflammation. Fatty acid composition, peroxide number and free fatty acidity analyzes of commercially available *C. gileadensis* balsam oil and investigation of its effect on cell viability. Commercially available *C. gileadensis* balsam oil fatty acid composition, peroxide number and free fatty acidity values were determined by the IUPAC IID19 method, and MTT cell viability tests were performed on L929 fibroblast and HeLa (human epithelial cervical carcinoma) cell lines. Commercially available *C. gileadensis* balsam oil did not exert cytotoxic effects on both L929 fibroblast and HeLa (human epithelial cervical carcinoma) cell lines and promoted the growth of cell lines. Due to its growth promoting feature on L929 fibroblast cells, which is a healthy cell line, this material can be used as a cell culture medium additive.

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INTRODUCTION

Commiphora gileadensis, also known as *C. opobalsamum*, a member of the *Burseraceae* family, is a small evergreen tree that grows widely in East Africa and tropical and subtropical regions such as Arabia and India (Hepper and Taylor, 2004; Iluz et al., 2010; Paparozzi, 2005; Vollesen, 1989; Almahbashi et al., 2019). *C. gileadensis* is still considered an important medicinal plant in the Middle East and particularly in Saudi Arabia, and is an aromatic herb traditionally used to treat pain, swelling and fever (Al-Sieni, 2014). It is also used to treat skin ulcers, empyrosis and wounds (Dai et al., 2001; Shang and Lu, 2007; Shen et al., 2012). Balsam oil is obtained from the trunk of this tree by wounding. In recent years, it has been reported that it contains cycloartane-type triterpenoid, an aliphatic alcohol glycoside, an eudesmane-type sesquiterpenoid, and a guaiane-type sesquiterpenoid (Shen et al., 2007; Shen et al., 2008; Shen et al., 2009; Xu et al., 2011; Xu et al., 2011a). Studies on the effects of commercially available *C. gileadensis* balsam oil on cell viability are limited.

In this study, fatty acid composition, peroxide number and free fatty acidity analyzes of commercially available *C. gileadensis* balsam oil were performed, and MTT cell viability tests were performed on L929 fibroblast and HeLa (human epithelial cervical carcinoma) cell lines.

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METHOD

C. gileadensis balsam oil

C. gileadensis balm oil, obtained by wounding the trunk of the *C. gileadensis* tree, was purchased from the local herbal store.

Determination of fatty acid composition

Fatty acid composition, peroxide number and free fatty acidity were determined by TUBITAK-MAM.

Cell culture

L929 fibroblast and HeLa (human epithelial cervical carcinoma) cell lines were used. DMEM + 2mM Glutamine + 10% Fetal Bovine Serum (FBS) and DMEM (High Glucose) + 10% FBS (Biological Industries) were used as cell culture medium, respectively. Cells were grown at 37 °C, 5% CO_2.

MTT-based cytotoxicity testing

C. gileadensis balsam oil sample was mixed into DMEM at concentrations of 180, 90, 45, 22.5 and 9 µg/mL. It was sterilized through a 0.22 µm filter, and the L929 and HeLa cell lines were used for MTT-based cytotoxicity testing. The L929 cell line and the HeLa cell line were seeded at 10^5 cells/mL in 96 well cell culture dishes. Cells were allowed to attach to the bottom in an incubator at 37 °C, 5% CO_2 for 24 hours. At the end of 24 hours, 20 µl of *C. gileadensis* balsam oil sample prepared at the above-mentioned concentrations was added to each well. MTT viability test (BioFroxx) was performed in an incubator at 37 °C, 5% CO_2 for 24 hours. 1% phenol solution was used as the positive control, only DMEM and DMEM F12 media were used as the negative control, and the experiments were performed as three independent replicates and the averages are presented for both L929 fibroblast and HeLa cell line. Photometric reading was performed at 570 nm. Results were calculated assuming the negative control is 100% viable.

RESULT

Fatty acid composition of *C. gileadensis* balsam oil

C. gileadensis balsam oil fatty acid composition, peroxide number and free fatty acidity values obtained as a result of the analysis are given in Tables 1, 2, 3, respectively.

Cell viability test

Analyzes of effect of *C. gileadensis* on cell viability were performed by MTT viability assay on L929 fibroblast and HeLa cell lines. Information on the mean absorbance and viability percentage values of the *C. gileadensis* balsam oil sample on L929 fibroblasts and HeLa cells at concentrations of 180, 90, 45, 22.5 and 9 µg/mL are given in Table 4, 5 and Fig 1, respectively.

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Table 1. *C. gileadensis* balsam oil fatty acid composition

Analysis	Result (%)	Method
Caproic acid (C6:0)	0.37	IUPAC IID19
Caprylic acid (C8:0)	12.60	IUPAC IID19
Capric acid (C10:0)	0.12	IUPAC IID19
Lauric acid (C12:0)	0.14	IUPAC IID19
Myristic acid (C14:0)	0.10	IUPAC IID19
Myristoleic acid (C14:1)	0.01	IUPAC IID19
Pentadecanoic acid (C15:0)	0.10	IUPAC IID19
Palmitic acid (C16:0)	15.50	IUPAC IID19
Palmitoleic acid (C16:1)	0.11	IUPAC IID19
Heptadecanoic acid (C17:0)	0.14	IUPAC IID19
Stearic acid (C18:0)	10.73	IUPAC IID19
Elaidic acid (C18:1n9t)	0.08	IUPAC IID19
Oleic acid (C18:1n9c)	33.36	IUPAC IID19
Linoleic acid (C18:2n6c)	0.56	IUPAC IID19
Arachidic acid (C20:0)	0.51	IUPAC IID19
γ-Linolenic acid (C18:3n6)	0.16	IUPAC IID19
cis-11-Eicosenoic acid (C20:1)	0.22	IUPAC IID19
α-Linolenic acid (C18:3n3)	0.01	IUPAC IID19
Behenic acid (C22:0)	0.22	IUPAC IID19
Lignoceric acid (C24:0)	0.09	IUPAC IID19

Table 2. *C. gileadensis* balsam oil peroxide number

Analysis	Result	Method
Peroxide value	Not dedected	meq active ISO 3960 oxygen/kg oil

Table 3. *C. gileadensis* balsam oil free fatty acidity

Analysis	Result **(%)**	Method
Free fatty acidity	0.18	The acidity based on the oleic acid content ISO 660

Table 4. Viability of L929 after exposure to *C. gileadensis* balsam oil measured by MTT assay

Concentration (µg/mL)	180	90	45	22.5	9	Negative control	Positive control
Average absorbance	2.44855	2.651	2.50895	2.07815	2.035775	1.0915	0.0859
Viability percentage	224.336	242.884	229.870	190.400	186.517	100.000	7.870

Table 5. Viability of HeLa after exposure to *C. gileadensis* balsam oil measured by MTT assay

Concentration (µg/mL)	180	90	45	22.5	9	Negative control	Positive control
Average absorbance	3.877	3.643	3.636	3.634	2.035775	3.716	0.136
Viability percentage	134.82	126.68	126.47	126.39	129.24	100.000	4.74

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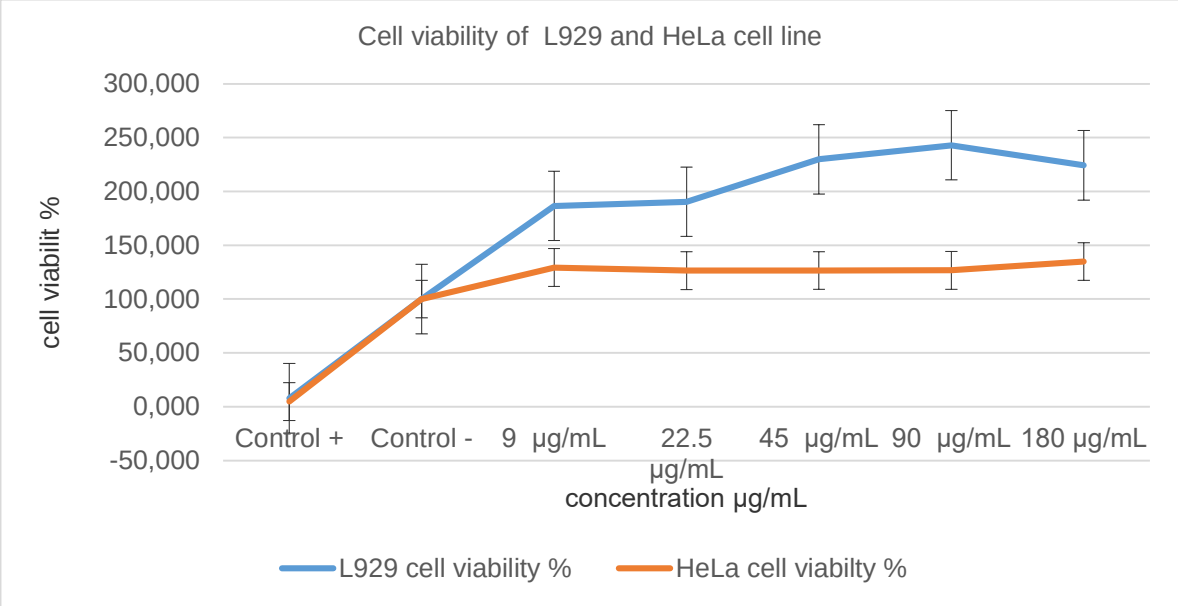

Figure 1. Viability of L929 and HeLa cell line following *C. gileadensis* balsam oil treatment of MTT viability assay

According to the results obtained, when the balm oil L929 fibroblast cell line was compared with the negative control group, it showed 186.5% viability at 9 µg/mL concentration, 190.400% viability at 22.5 µg/mL concentration, 229.870% viability at 45 µg/mL concentration, 242.884% viability at 90 µg/mL concentration, respectively. It showed 224.336% viability at 180 µg/mL concentration.

Therefore, it did not have a toxic effect on cell viability at all concentrations and promoted cell viability. The same effects on the HeLa cell line, when compared with the negative control group, were 129.24% viability at 9 µg/mL concentration, 126.39% viability at 22.5 µg/mL concentration, 126.47% viability at 45 µg/mL concentration, and 126.68% viability at 90 µg/mL concentration, and 134.82% viability at180 µg/mL concentration, respectively.

DISCUSSION

Fatty acid content analysis of *Commifera* species in the literature has been reported as linoleic, oleic, stearic, and palmitic acids in the seed of *C. mukul* (Hanuš et al., 2005). In the core of *C. wightii* species, it is reported as capric acid 3.50%, myristic acid 14.51%, palmitic acid 6.68%, stearic acid 4.70%, arachidic acid 3.18%, behenic acid 14.05%, myristoleic acid 1.34%, palmitoleic acid 12.07%, oleic acid 14.15%, eicosenoic acid 0.11%, linoleic acid 22.34% and alpha linoleic acid 1.37% (Patel et al., 2009). In this study, caprylic, palmitic, stearic, and oleic acids were detected in the balsam oil of *C. gileadensis* at the rates of 12.60%, 15.50%, 10.73%, and 33.36%, respectively.

Plants with a broad spectrum of chemical compounds have the potential to form many therapeutic components for various diseases and also serve as a pool for the development of alternative pharmacological products (Bomfim et al., 2021). Since ancient times, people have either obtained herbal extracts for various purposes by traditional methods or bought them from local stores as it is more common today. Among these purposes, skin care is at the forefront. The

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reliability of these extracts, which are purchased and used in this way, is also important in terms of personal health.

C. gileadensis has been used extensively in the Middle East for centuries, both as a perfume and in traditional medicine (Hepper and Taylor, 2004; Iluz et al., 2010; Wineman et al., 2015). In addition to being used as an antiseptic agent by some tribes in Oman, *C. gileadensis* is also used ethnobotanically in the treatment of skin diseases such as inflammation and eczema (Iluz et al., 2010).

It has been reported that it has an anti-proliferative effect on cancer cell lines in studies carried out with *C. gileadensis* extracts prepared under laboratory conditions. In a study conducted by Shen et al. in 2007, it was shown that *C. gileadensis* secondary metabolites have an antiproliferative effect on human prostate cancer cells (Shen et al., 2007). In another study with *C. gileadensis* sap extract, immortalized human keratinocyte cells and in A431 cells were used and it was reported that it had a selective cytotoxic effect (Wineman et al., 2015). In another study, the cytotoxicity of *C. gileadensis* L bark methanolic extracts against HELa and A-549 was investigated and it showed moderate dose-dependent cytotoxic activity in these cells. It has been reported that it exhibits weak cytotoxic effects in MCF-7 HepG2, HCT-116, HEP-2 and PC-3 cell lines (Almahbashi et al., 2019).

The commercially available balsam oil used in this study did not show cytotoxic effects on the HeLa cancer cell line. It did not show toxic properties on L929 healthy cell lines, and it promoted cell viability.

When considering application areas such as tissue engineering or cell culture, materials that promote cell viability or that are not toxic to cell viability are important. As a result, *C. gileadensis* balsam oil, which has no toxic effect for maximum cell productivity and has been observed to promote cell proliferation, has the potential to be used for this purpose.

CONCLUSION

This study showed that commercially available *C. gileadensis* balsam oil has no toxic effect on L929 fibroblast and HeLa cell lines. Due to its growth promoting feature on L929 fibroblast cells, which is a healthy cell line, this material can be used as a cell culture medium additive by further research. *C. gileadensis* balsam oil is thought to have the potential to be used in cell culture production of pharmacological products for both pets and humans.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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