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Evaluation of the cytotoxic effects of *Boswellia sacra* and *Origanum majorana* against mice lymphocytes and RD cell lines *in vitro*

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Objective Herbs and plant extracts were common selected substances that has medicinal use and was used long time ago to protect or to heal people in need, many of these plants has wide effect on different biological systems including humans, animals, and bacteria. This study was about the potential effect of *Boswellia sacra* and *Origanum majorana* as an anti-tumor agent.
Methods Two plants were extracted resulting aqueous or alcoholic extractions depending on the solvent that is used for extraction. Soxhlet apparatus had used for methanolic extraction while stirring distilled water were used for aqueous extraction on a magnetic stirrer.
Results Mice RD (ATCC® CCL-136™) cells inhibited with a different pattern, five concentrations used in the study. The 50 mg/ml of all extracts exhibit almost the same inhibition activity, whereas the activity of aqueous *B. sacra* extract was more active at 25, 12.5, 6.25 and 3.12 mg/ml in comparison with methanolic *B. sacra* extract which had the most inhibition activity by killing most of studied cells at 6.25 mg/ml. On the other hand aqueous *O. majorana* extract exhibits an inhibitory activity higher than methanolic extract after the 50 mg/ml, which may due to the polar constituent of extracted substances while in 12.5 mg/ml, was the best of relatives. The effect of methanolic extract decreased in diluted concentrations**.** Aqueous extract of *B. sacra* had a superior activity compared with methanolic extract, which took the same effectiveness with less inhibitory effect, but in 6.25 mg/ml was the point where methanolic had the best activity among all extracts.
Conclusion This study suggests using aqueous extract instead of methanolic extracts that affects and inhibits the cancerous cell line that would be a good replacement of other drugs on patients.
Keywords *Boswellia sacra*, *Origanum majorana*, anti-cancer, cytotoxic, MTT, aqueous extraction, methanolic

Introduction

Medicinal plants are important therapeutics, the use of those that are native to among traditional system of medicine that leads to alternative natural substances uses and applications of these plants. It has been used as precursors for different medicinal chemicals or as herbal derived drugs, many extraction procedures have used for years to extract and test the active plant ingredients, which had used as drugs and drugs replacements.[1]

The essential oils possess antibacterial, antifungal, antiviral, antioxidant and wide spectrum of pharmacological activities. The genus *Boswellia* has more than 20 species occurring in the arid regions in Arabic regions, West Africa, India, China, Somalia, and Madagascar.[2,3] Frankincense (Kinder) is an aromatic and milky resin harvested from trees of the genus *Boswellia* spp.,[4] it is also known in as Luban in Arabic, it has been used in cosmetics and perfumes.[5] *Boswellia* sp. includes *Boswellia sacra* from Oman and Yemen, *Boswellia carterii* from Somalia, and *Boswellia serrata* from India and China. It is used in incense and fumigants.[6,7]

Four different grades and oil yield found in Oleo-Gum Resin of *B. sacra*, the color grades from dark color in Shaabi, to shathari, Najdi (Pale yellow) and Hoojri (light color). Essential oil from each grade are colorless with a conifer-like fragrance but they differ in oil content percentage (v/w), all grades have bactericidal activity.[8]

Boswellia sacra has great medicinal uses in the healing of toothache, bronchitis, tumors, leukoderma, scurvy, and scabies. Generally composed of about 5–9% essential oil, 65–85% alcohol-soluble resin, and the remaining water-soluble gum[9] and has different plant important constituents,[10] and *B. sacra* oils has an antibacterial activity against *Staphylococcus aureus, Klebsiella pneumoniae*, *Escherichia coli, Proteus mirabilis*, *Salmonella paratyphi*, *Enterobacter faecalis*, *Salmonella typhi*.[12]

Crude essential oil of *B. sacra* gum resins might be a useful alternative therapeutic agent for treating patients with pancreatic adenocarcinoma.[13] It is also shown to reduce cerebral edema and potential anti-cancer activity in patients irradiated for brain tumors.[14] *Boswellia sacra* essential oil induces breast cancer cell-specific cytotoxicity.[15]

Extracts from *Boswellia* sp. have shown to possess antifungal against clinical animal isolates,[16,17] gastric and hepatic disorders,[18] antiglycation and antioxidant activities,[19] and tumor suppression,[15] these results suggest that resins from *Boswellia* sp. contain active ingredients that modulate important biological and health supporting activities.

Using various approaches, the resin showed a pentacyclic triterpenic compounds, in a chemical point of view. *Boswellia carterii* and *B. sacra* especially characterized by the presence of lupeolic acid, boswellic acids,[20] nine tetraterpines,[21] with high content of the monoterpenes and less constitutes of sesquiterpenes obtained from frankincense of *B. sacra* Flueckiger along with four known compounds.[22]

Mardagosh [The genus *Origanum majorana* (L.)] an aromatic, herbaceous and perennial plant belongs to the family Lamiaceae. It is used as a flavoring and herbal spice, known commercially and widely used in flavoring food and perfumery.[23]

Origanum majorana is the main source for terpinen-4-ol and many valuable components such as δ-terpinene, *p*-cymene,

sabinene, α-terpineol, α-phellandrene, and β-caryophyllene. In addition, this plant has strong activities and could potentially a natural source of phenolics and antioxidants.[24,25]

Leaf powder and essential oil of *O. majorana* L. confirmed presence of number of phytoconstituents such as carbohydrates, proteins, amino acids, saponins, flavonoids, alkaloids, phenolic compounds, vitamin C and tannin in aqueous extract whilst essential oil possessed only flavonoids, phenols and volatile oils.[26]

Results showed an antibacterial effectiveness of *O. majorana* essential oil and supported the possibility of their use as a source of alternative antimicrobial agent.[27]

The ethanol extract of *O. majorana* has strong microbicidal property and superiority over commercial microbicides.[28] The essential oils obtained from *O. majorana* has an antifungal activity,[17,26] it also showed bioactivity against bacteria *S. aureus*, *Listeria monocytogenes*, *Salmonella enteritidis*, *Salmonella typhimurium*, *Bacillus* sp. and *Streptococcus* sp., *E. faecalis*, *E. coli*, *Listeria ivanovii*, *Listeria inocula*.[29–32] Therefore, this article aimed to study a potential effect of *B. sacra* and *O. majorana* as an anti-tumor agent.

Materials and Methods

Plant material

Fresh plant materials bought in 2017 from a traditional market in Erbil, Iraq, dried and grinded to fine particles.

Plant extractions

Preparation of the methanolic extracts

Using Soxhlet, the dried and powdered branch (50 g) was extracted with 350 ml of methanol using a Soxhlet extractor for 7 h at a temperature of 64°C not exceeding the boiling point of the solvent (70°C).

Aqueous extraction

A dry and powdered *B. sacra* and *O. majorana* (20 g) were added to 200 ml of distilled water, then mixed using magnetic stirrer for 1 h in room temperature, mixture separated using centrifuge at 3000 rpm for 15 min, supernatant collected and dried using oven at 40°C.[34]

Cell Lines and Culture Conditions

Mice RD cell line: Rhabdomyosarcoma with human muscle origin.

Cells generously supplied from the lab of Animal cell culture at the Biotechnology Research Center Al-Nahrain University, Baghdad, Iraq.

The base medium for this cell line was Dulbecco's modified Eagle's medium (Gibco, USA) to make the complete growth medium, fetal bovine serum (Gibco, USA), was added to a final concentration of 10%.

Mouse lymphocytes

Cells were isolated from spleen of adult cultured mouse and maintained at the animal cell culture lab, Biotechnology Research Center, AL-Nahrain University, Baghdad, Iraq. Cells were sub-cultured and maintained with RPMI 1640 medium (Gibco, USA) supplemented with 10% fetal bovine serum (Gibco, USA).

MTT assay

MTT Kit (Thermo Fisher, USA), the MTT [MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MW = 414, Component A), 10 vials, each containing 5 mg] dissolved in 1 ml PBS, and DMSO as a solvent.

Cells (RD and mouse lymphocytes) both seeded into a 96-well plates in a cell density from 5000 to 10,000 cells/well incubated over night to ensure the cells growth and no contamination. Extract concentrations prepared (concentrations) filtered and added directly to the cells and incubated for 48 h.

MTT protocol

For adherent cells, remove the medium and replace it with 100 µL of fresh culture medium.[33] For non-adherent cells, centrifuge the microplate, pellet the cells, carefully remove as much medium as possible and replace it with 100 µL of fresh medium. Add 10 µL of the 5 mM MTT stock solution to each well. Include a negative control of 10 µL of the MTT stock solution added to 100 µL of medium alone. Plates were incubated at 37°C from 2 to 4 h, DMSO added 50 µL to each well and mixed thoroughly with the pipette then incubated at 37°C for 10 min, each sample was mixed again and the absorbance read at 540 nm.

Cell Viability

Cells viability done using percentage equation:

$$\%\ \text{Viability} = \frac{\text{Treated cells}}{\text{Control}} \times 100\%$$

Results and Discussion

Plant extracts play a good role in suppression of many biological systems like bacteria, fungi… etc., those extracts differs from each other in the constituent and the type of active compounds that will affect studied organisms.[1]

Boswellia sacra were capable of suppressing viability and inducing apoptosis of a panel of human pancreatic cancer cell lines in both water and methanolic extracts as they may induce specific cytotoxicity of breast cancer cells by repressing signaling pathways and cell cycle leading to the suppression of cellular network formation and disruption of spheroid development of breast cancer cells.

It also induced apoptosis fragmentation of genomic DNA demonstrated which was similar as shown by Suhail et al.[14]

In this experiment, two plants were extracted using water and methanol for extraction, and then applied on RD mouse cancer cells to investigate the possible effect of extracts on those cells. Different patterns of activity shown in Figs. 1 and 2, at a concentration of 50 mg/ml of aqueous and methanolic extracts.

All extracts exhibit an inhibition activity, whereas the activity of aqueous *B. sacra* extract was more active at concentrations 25, 12.5, 6.25 and 3.12 mg/ml, in comparison with methanolic *B. sacra* extract which had the most inhibition activity by killing most of the studied cells at 6.25 mg/ml. This results confirmed early studies, who found that Crude essential oil of *B. sacra* gum resins might be a useful alternative therapeutic agent for treating patients with pancreatic adenocarcinoma and breast cancer cell-specific cytotoxicity.[13,15]

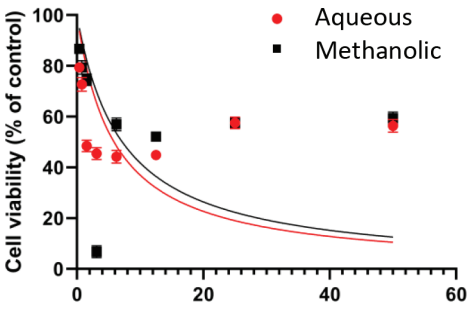

Fig. 1 **Cell viability average of *B. sacra* extract.**

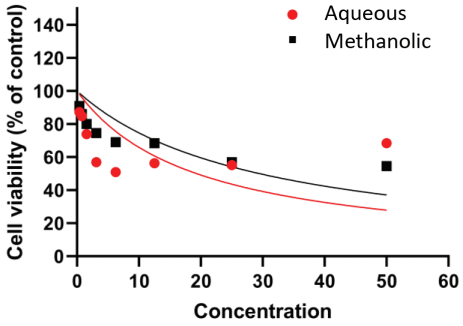

Fig. 2 **Cell viability average of *O. majorana* extract.**

On the other hand, aqueous *O. majorana* extract exhibits an inhibition activity higher than methanolic extract beyond second concentration (25 mg/ml). This may due to the polar constituent of the extracted active compound, and the 12.5 mg/ml was the best of relatives while the effect of methanolic extract decreased in the other concentrations. Aqueous extract of *B. sacra* had a superior activity compared with methanolic extract that have the same curve but with less inhibitory effect, but in 6.25 mg/ml was the point where methanolic had the best activity among all extracts. The reason may be due to the fact of *O. majorana* has many valuable phytoconstituents with strong activities and could potentially be used as natural source of phenolics and antioxidants in aqueous and essential oils extract so its potentially active substances.[24–26]

Conclusion

Using aqueous extract instead of methanolic extracts as it inhibits the cancerous cell line that would be a good replacement of other drugs and non-toxic as methanol.

Conflicts of Interest

None.

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