Journal of Scientific & Industrial Research
Vol. 78, July 2019, pp. 419-425

# Assessment of *In Vitro* Antimicrobial and Anti-breast Cancer Activities of Extracts Isolated from Desert Truffles in Saudi Arabia

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*Received 21 August 2018; revised 14 April 2019; accepted 03 June 2019*

Truffles are consumed worldwide as a type of precious food. Desert truffles are characterized by their growth under extreme soil and climate conditions. They have numerous nutritional and medicinal applications. Desert truffles have been shown to exhibit various biological activities. During the present work, we identified two truffle types collected from Riyadh Province, Kingdom of Saudi Arabia, as *Tirmania nivea* and *Terfezia claveryi*. Their extracts showed significant antimicrobial activity against *Bacillus subtilis*, while the activity was less obvious against *Escherichia coli*. Hexane and ethyl acetate extracts of both types showed a dose-dependent effect against MCF-7 cancer cells, where their highest toxicities ranged from 91-93%. The lowest effective $IC_{50}$ values were 378.9±0.96 and 215.8±0.92 µg/mL for *T. nivea* and *T. claveryi*, respectively.

**Keywords:** Desert truffles, Antimicrobial, Anticancer, MCF-7, Solvent extraction

## Introduction

Cancer is one of the most problematic diseases affecting human life. World Health Organization (WHO) rates cancer as the second worldwide leading death cause with an estimated 9.6 million deaths in 2018[1]. Breast cancer is considered as the second most common cancer type, with 2.09 million cases, and is responsible for the death of 627,000 cases annually[2]. Currently, chemotherapy and radiotherapy are the most widely applied treatments in cancer therapy[3]. However, such treatment methodologies result in severe side effects on patients, ranging from nausea, allergic reactions, decreased immune response to bleeding and toxicity. Moreover, patients suffer from the chance of cancer being translocated into other body organs, with consequent complications. Therefore, the search for relatively mild treatments is still the main goal for researchers. In such context, natural resources provide a better alternative for exploring bioactive compounds and molecules. Compounds derived from natural resources (plants and microorganisms) are non-toxic to patients and have little if no side-effects at all[4].Truffles are used as a form of traditional food. Also, they are largely consumed worldwide due to their high nutritional qualities[5]. They are hypogeous macrofungi growing under the surface with no gills or stalks[6]. They contain adequate quantities of carbohydrates (60%), proteins (20-27%), fats (3-7%), fibers (7-13%) and 2-5% ascorbic acid. Desert truffles belong mainly to the genera *Terfezia, Delastreopsis, Balstonia, Delastria, Leucangium, Mattirolomyces, Phaeangium, Picoa, Tirmania,* and *Tuber,* and have been microbiologically classified into 6 Ascomycete families. They have been widely distributed in deserts of the Middle Eastern countries in Africa and Asia. In terms of biological activities, truffles have been found to exhibit a wide range of bioactivities[4]. They have been reported to possess potent antioxidant, antimicrobial, antiulcer as well as anticancer activities[4,6], and they can act as immunomodulators[7-9]. Furthermore, they have been found also to act as anti-inflammatory as well as having a cholesterol and sugar lowering effect[10]. Although truffles have been widely consumed as dietary food as well as medical treatment in some

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traditional and folk practices, however, to our knowledge, their potential as a source for bioactive compounds has not been fully investigated. Until now, there is little information available on bioactive studies concerned with truffles found in Saudi habitats. Therefore, the current study was designed to investigate the potential of different extracts from two desert truffles collected from Riyadh province, Saudi Arabia, as antimicrobial and anti-breast cancer agents.

## Materials and methods

### Sample collection, identification and extraction

Different truffle samples were collected from two locations around Riyadh, Kingdom of Saudi Arabia; 25°39'06.7"N 46°34'19.1"E and 23.3919489°N 46.2978111°E. Upon collection, samples were classified, photographed, washed with distilled water, and then were air-dried at 40°C for 24 h. Dried samples were grounded with an electric mixer till powder formation. Extraction mixtures (10:1, v/g) were prepared using different solvents; methanol, methanol 70%, water, ethyl acetate and hexane), then agitated fat 250 rpm for 24 h, then filtered with sterile cotton, centrifuged at 4000 rpm for 10 min. The supernatant is then taken and evaporated with rotary evaporator at 45°C and 150 rpm. From the finally obtained precipitates, stock solutions were prepared in DMSO, which were used to prepare series of serially diluted working solutions at different concentrations. Identification was used using general mycology taxonomic key with the help of specialized scientists in the field of Microbiology.

### Microorganisms and cell line

Microbial strains used in the current work included the Gram-positive bacteria *Staphylococcus aureus* NRRL B-313 and *Bacillus subtilis* NRRL B-543, and the Gram-negative bacteria *Escherichia coli* JM DSM 3949 and *Klebsiella pneumonia.* Breast cancer cell line MCF-7 was obtained from Sigma-Aldrich Chemical Company, NJ, USA.

### Antimicrobial assay

Antimicrobial activity of different prepared extracts was evaluated using the modified agar diffusion method[11]. Microorganisms were propagated using nutrient agar at 37°C. Before the assay, microbial strains were grown in nutrient broth media for 6 hours on a rotary shaker at 150 rpm at 37°C. When cells are in the exponential phase, plates containing agar media were inoculated with cells to insure a final cell concentration of 0.015/mL. When the agar plates solidify, a sterile cork borer is used to make 10 mm wells, then and 0.1 mL of each of the serial dilutions was pipetted into each well. Plates were then incubated for 24 hours at 37°C and then the diameter of the developed resulting inhibition zones was measured.

### Cultivation of MCF-7 cells and assay of cytotoxic activities

MCf-7 cancer cells were grown in DMEM supplemented with 10% serum, 100x, 1% antibiotic solution, in a humid-$CO_2$ incubator (ShelLab, USA) at 5% $CO_2$, 37°C. On the day of assay, cells were detached from growing surface, centrifuged and washed twice with sterile PBS buffer solution. Viable cell concentration was determined using Trypan Blue exclusion[3]. Grown cells were treated with different working solutions of truffle extracts ranging from 0.0 to 1000 µg/mL. Afterwards, the cytotoxic activities were determined screened using standard MTT assay. Control wells were only treated with DMSO ≤ 0.5%). The assay was performed as we previously described[4,12]. Prepared cells were seeded in 96 well culture plates with a final concentration of $10^4$ cells/100 µl/well, and then were grown for 24 h. Afterwards, the medium was aspirated and substituted with fresh medium supplemented with different concentrations of different prepared extracts, and then grown further for 24 h. Plates were microscopically examined for morphological changes. Then, MTT (10 µl, 5 mg/ml in PBS) was added for 4 h, and the developed formazan crystals were dissolved using 200 µl of DMSO. Absorbance was measured at 550 nm. Cell viability was calculated in relation to the control. Concentrations producing 50% decrease in cell growth were taken as $IC_{50}$ values.

### Statistical analysis

Statistical analysis for all data was analyzed as a completely randomized design using One Way Analysis of Variance (ANOVA) followed by Duncan test. Comparisons will be considered significantly different at $p \leq 0.05$. Results are expressed as a mean ± standard Deviation (S.D).

## Result and Discussion

### Identification of truffle samples

Two main truffle types were collected during the study. The samples are traditionally known as Zubaidi (white-cream) and Khlasi (black-dark brown) truffles. They were morphologically characterized and

identified at KSU herbarium with the help of mycological taxonomic keys as *Tirmania nivea* and *Terfezia claveryi*, respectively (Figure 1A).

**Antimicrobial activities of different extracts of saudi truffles**

The antimicrobial activities of methanolic and ethyl acetate extracts of the two collected types were evaluated against G+ve and G-ve bacterial strains at different concentrations (1, 10 and 20 mg/mL).

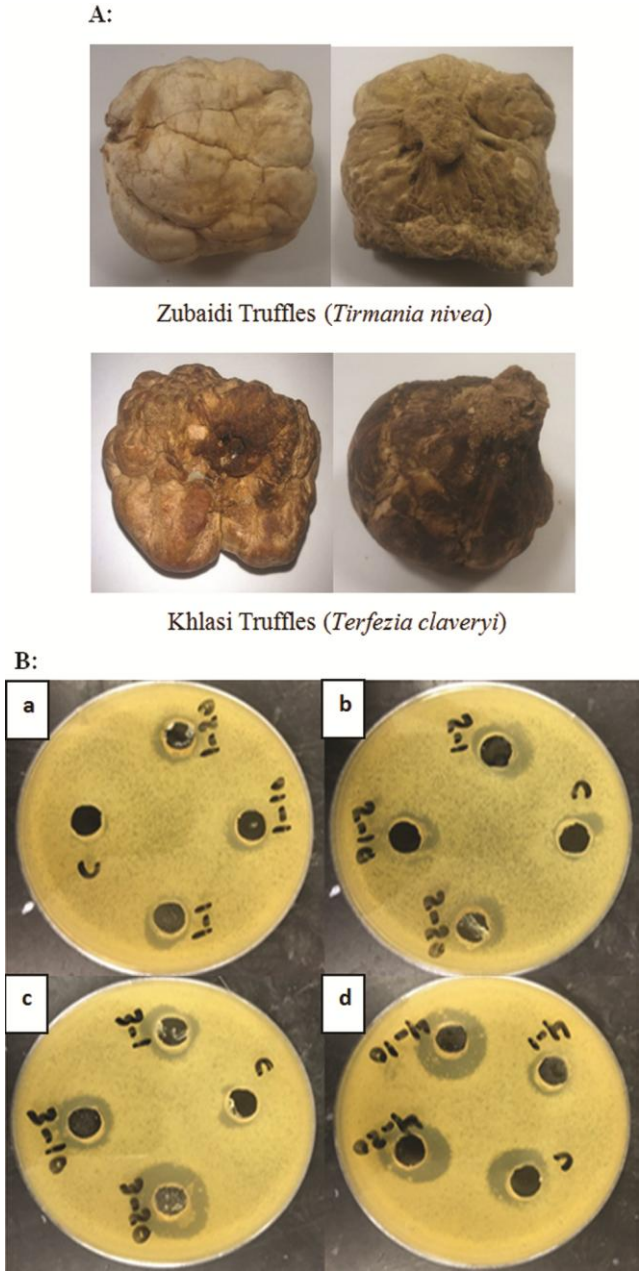


Fig. 1 — A: Truffle samples isolated and identified from Riyadh province, KSA. B: Antimicrobial activities of different extracts of truffle samples against *B. subtilis* (a: *T. nivea*, MeOH; b: *T. claveryi*, MeOH; c: *T. nivea*, EtAc; d: *T. claveryi*, EtAc).

Screening results showed that among tested G-ve bacteria, only ethyl acetate extract of *T. nivea* was effective against *E. coli* at 10 and 20 mg/mL concentrations, where the obtained inhibition zones recorded 15.0 and 18.3 mm, respectively (Figure 1B). Concerning G+ve bacteria, *S. aureus* was not affected by different concentrations of different extracts tested. On the other hand, all extracts tested showed good inhibition zones at all evaluated concentrations against *B. subtilis* (Table 1). Natural resources, plants and microorganisms, have been long searched for the presence of bioactive compounds[13,14]. Compounds with diverse biological activities have been found and isolated from microorganisms. Desert truffles are symbiotic hypogeous Ascomycetes, which are believed to develop under certain hard climatic conditions. Therefore, search has continued to extract and isolate compounds with interesting bioactive compounds. Our results showed that *B. subtilis* is the most affected bacterial strain by the different truffle extracts. These results are in agreement with those reported by Malik *et al.*[15], who investigated the role of aqueous extracts of *Terfezia* spp. against both Gram-ve and Gram+ve pathogenic microorganisms. They attributed their findings to the protein-nature of compounds present in truffle extract, which may have an inhibitory effect of both types of pathogenic bacterial strains. On the other hand, Hamza *et al.*[16] found that their methanolic and cholorform extracts of *Tirmania nivea* obtained from Moroccan deserts have antimicrobial activities against both Gram-ve and Gram+ve pathogenic strains superior to the aqueous extracts. They explained their results on the basis that polar solvent extract more polar compounds found in the truffle tissues. Additionally, we previously reported that the antimicrobial effect of truffle extracts was mainly related to the production of low-molecular weight peptide antibiotics[6].

**Anticancer activities of different extracts of Saudi truffles**

The cytotoxic effects of different solvent extracts of collected truffle types (*T. nivea* and *T. claveryi*) were evaluated against MCF-7 cells at different concentrations ranging from 0.0-1000 µg/mL. Results (Figure 2 and figure 3) showed similar trends of anticancer activities of different solvent extracts of both truffle types. This was confirmed by the statistical analysis which showed that there was no significance between the two truffle types ($p = 0.07$). Furthermore, the effect of different solvents on the cytotoxic activity against MCF-7 sowed very high

Table 1 — Antimicrobial activities of different solvent extracts of truffle samples at different concentrations (mg/mL)

| Extract | *K. pneumonia* | | | *E. coli* | | | *S. aureus* | | | *B. subtilis* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1.0 | 10.0 | 20.0 | 1.0 | 10.0 | 20.0 | 1.0 | 10.0 | 20.0 | 1.0 | 10.0 | 20.0 |
| A | - | - | - | - | - | - | - | - | - | 12.0 | 14.0 | 15.0 |
| B | - | - | - | - | 15.0 | 18.3 | - | - | - | 16.6 | 17.0 | 24.3 |
| C | - | - | - | - | - | - | - | - | - | 14.0 | 15.6 | 17.0 |
| D | - | - | - | - | - | - | - | - | - | 13.3 | 20.3 | 21.6 |

A: *T. nivea*, MeOH; B: *T. nivea*, EtAc; C: *T. claveryi*, MeOH; D: *T. claveryi*, EtAc

A*: Zubaidi Truffles (Tirmania nivea)*

*B: Khlasi Truffles (Terfezia claveryi)*

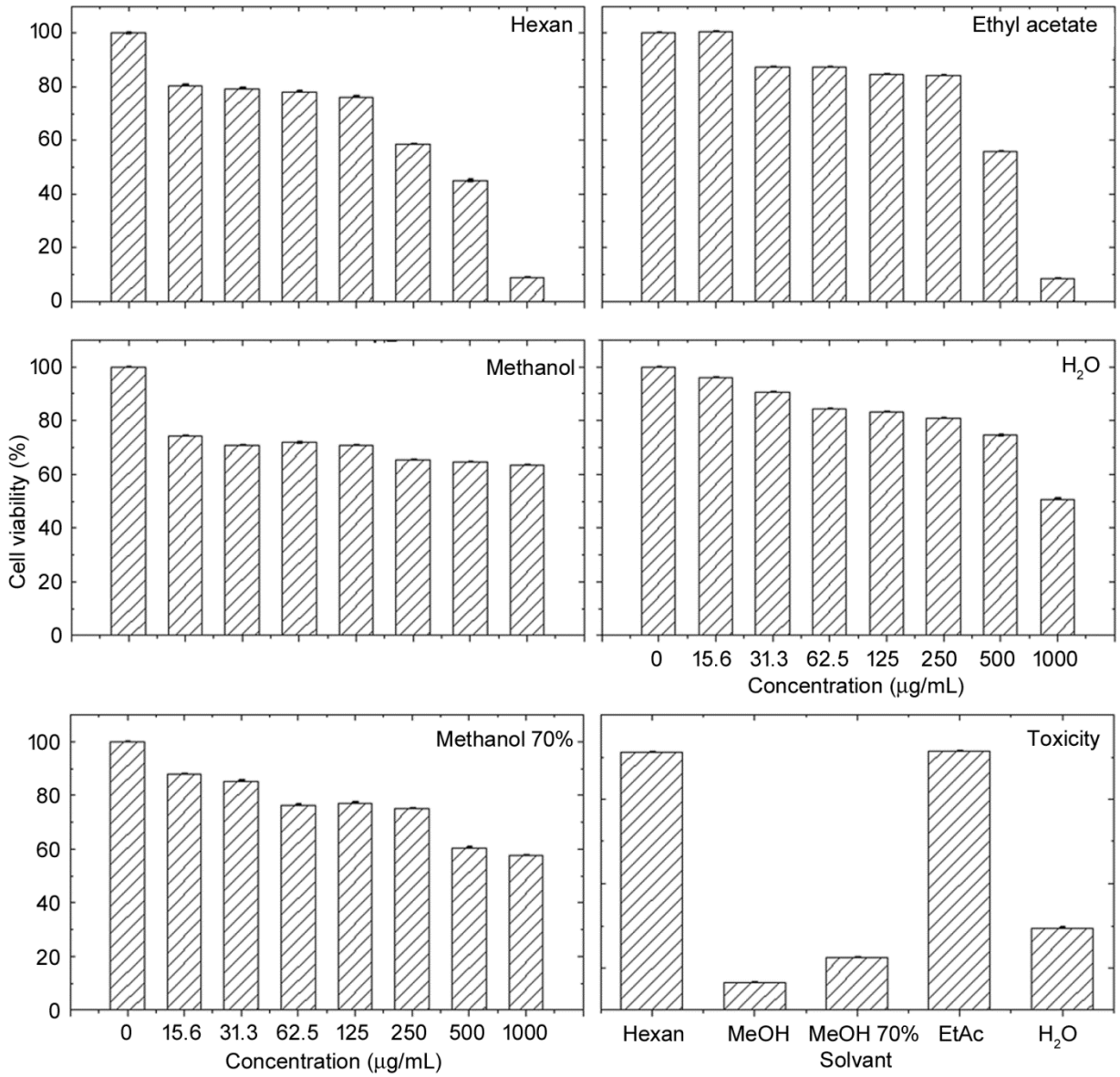


Fig. 2 — Cytotoxic activities of different solvent extracts of *T. nivea* against MCF-7 cells. Data are expressed as means±SD.

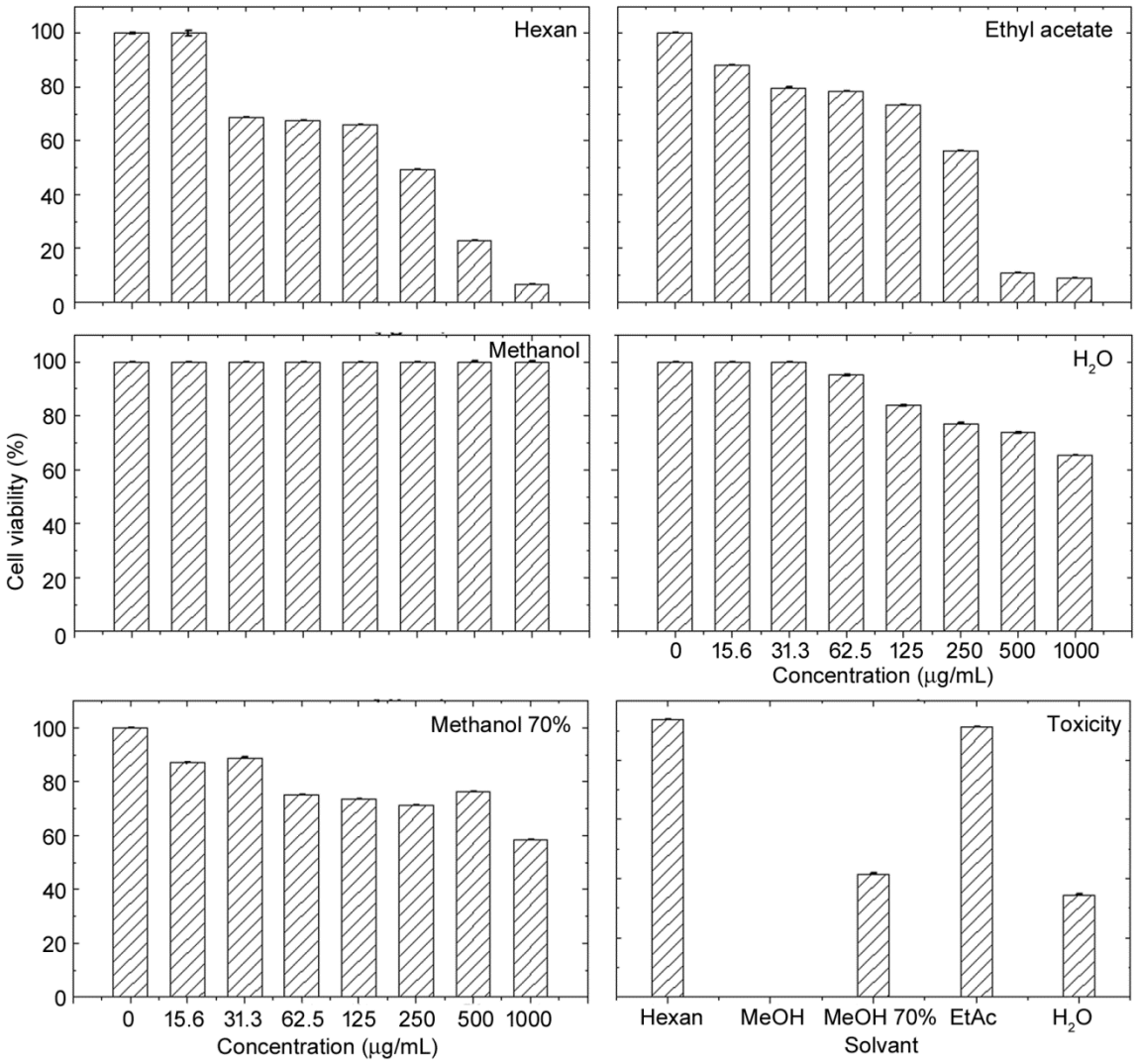


Fig. 3 — Cytotoxic activities of different solvent extracts of *T. claveryi* against MCF-7 cells. Data are expressed as means±SD.

significant results ($p$ = 0.0001). Increasing extract concentration resulted in a noticeable increase in cytotoxic activities, until reaching the maximal activity against MCF07 cells at the highest applied concentration (1000 µg/mL). Furthermore, it can be seen that the methanolic extract of khlasi truffle (*T. claveryi*) exhibited no cytotoxic activities against MCF-7 cells. Concerning maximal toxicity obtained on MCF-7 cells, results (Figure. 2F and 3F) showed that the order of maximal toxicities obtained for hexane and ethyl acetate extracts were similar for both truffle types (91.18±0.012 and 91.31±0.001%, respectively for *T. nivea* and 93.6±0.002 and 91.13±0.005%, respectively for *T. claveryi*). These results were also confirmed by the obtained $IC_{50}$ values, which were only obtained for the hexane and ethyl acetate extracts for both truffle types. The obtained $IC_{50}$ values ranged from 378.9±0.96 to 558.37±0.91 µg/mL for *T. nivea* hexane and ethyl acetate extracts, respectively, and from 215.8±0.92 to 282.71±0.93 µg/mL for *T. claveryi* hexane and ethyl acetate extracts, respectively. The other solvent extracts did not show any calculable $IC_{50}$ values, since their highest applied concentration did not reduce cell viability more than 50%.Results of cytotoxic activities of different extract of both types of truffles have clearly indicated that there is great a similarity between both types. Moreover, MCF-7 cells reacted differently towards different extracts of both types. These results are in good accordance with our previously published work on anticancer activities of different microbial metabolites as well as bioactive

molecules[4,17,18]. These results are explained on the basis that cancer cells react differently towards bioactive agents due to different interaction between cell membrane receptors and active compounds. Additionally, the antitumor activities of truffle extracts have been associated with the presence of fungal polysaccharides[19]. Polysaccharides form *T. claveryi* have been reported to greatly inhibit the cell proliferation of EAC, PC3, MCF-7 cells as well as human brain carcinoma[5,20]. Generally, different solvents were expected to exhibit different bioactive potential against MCF-7 cells, since they tend to extract different compounds found in truffle tissues depending on variability in solubility and polarity of compounds[21].

## Conclusion

During the current study, two truffle types were collected from Riyadh Province, Saudi Arabia, and were identified as *Tirmania nivea* and *Terfezia claveryi*. Secondly, their extracts proved to be potent against Gram+ve (*B. subtilis*) and Gram-ve (*E. coli*) pathogenic bacteria. In terms of anti-breast cancer activities, both hexane and ethyl acetate extracts were found to be most effective, while methanolic, 70% methanolic and water extracts were less effective against MCF-7 cancer cells. These results can be considered as the first report dealing with the effect of different solvent extraction on the anticancer activities of truffles found in Saudi habitats. Further studies are required for bioactive compound isolation and identification.

## Acknowledgments

Authors are thankful for the Deanship of Scientific Research, King Saud University, for funding the work through Vice Deanship for Scientific Research Chairs.

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