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Cytotoxicity and Antioxidant Determination of the *Juglans regia* L. Shells extract

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ABSTRACT
The history of the *Juglans regia* L. tree dates back to 7000 B.C. in Asia, making it one of the earliest trees from which people have ever harvested items(Vahdati, 2019). The area of this species' native distribution is unknown because of how long it has been cultivated. It is thought to be indigenous to Central Asia and the Mediterranean region (southern Europe and western Asia). According to fossil evidence, the latter nation's Western Himalayan ranges in Kashmir, Tajikistan, and Kyrgyzstan are where it originated (de Rigo, 2016). Different portions of walnuts offer curative or preventative qualities (Mutha, 2015). Medicine, many underestimate the value of walnuts as an important medicinal plant(Delaviz, 2017).Objective: This study evaluated antiproliferative and antioxidant activities of walnut.Context: Cancer chemopreventive action of walnut [Juglans regia L. (Juglandaceae)] has been explored.The antioxidant activity of walnut shell extract (JRSE) was studied using the DPPH assay.*Juglans regia* shell extract(JRSE) showed antioxidant activity against DPPH free radicals.Medicinal plants can be candidate as a common alternative for cancer treatment or act as synergistic with chemotherapy according to native plants in each country. JRSE was assessed against two cancer cell lines including prostate cancer cells PC3, lung cell line A549, that were compared to two normal cell lines including MCF10A normal breast cells and l929 normal mouse cells. According to MTT assay, JRSE showed toxicity levels close to each other for the four cell lines, ranked from highest to lowest cytotoxicity(256.252 for A549 cancer cells, 313.96 for normal L929 cells, 360.64 for PC3 cancer cells and 430.03 for normal MCF10A cells µg/ml) depending on IC50%.
Keywords: antioxidants, cytotoxicity, *juglans regia* shell extract.

INTRODUCTION

Currently, cancer ranks first or second among the causes of early death in the majority of the world's nations, from east to west .Due to demographic shifts, population expansion, and aging populations around the world over the next 50 year (Soerjomataram, 2021). The most prevalent cancers include prostate, breast, lung, colon, and rectum cancers (Bray, 2018). Plants have a wide variety of chemicals and adaptable biological characteristics that are ideally suited for their use as combination therapies to lessen the negative effects of cancer treatment (Liu, 2021). Numerous mechanistic investigations have furthermore supported the significance of phytochemicals in the fight against widespread malignancies (Khan, 2022). By generating cell cycle arrest, apoptotic states, down-regulating anti-apoptotic factors, and inhibiting the growth and proliferation of cancer cells, these naturally occurring phytochemicals may have anti-cancer effects (Asemi, 2019). *Juglans regia* L., often known as the common walnut, is a tough tree that has existed on our planet for a very long time (Sharma, 2020). Ni et al.(2022) found a positive relationship between walnut consumption and the prevention of several chronic diseases, including cancer. This is mostly because of the bioactive compounds that the plant's various components contain. The fruit walnut contains the majority of the important nutrients that people require for a balanced diet .The reason being that, like other hard-shelled fruits, walnuts are actually seeds, and all seeds are extremely rich in nutrients (Karadeniz, 2015). According to previous studies, walnuts contain many effective compounds and antioxidants that can be used as a treatment or to help reduce the side effects of some chemical treatments.

METHODS

Walnut shell Collection and Preparation.

With a nut cracker, the walnut shells were manually removed. They were then carefully cleaned, rinsed, dried, and ground into minute pieces using an electric grinder. Then it was sifted through a sieve the powder was then collected in airtight nylon bags and maintained in the laboratory at room temperature until it was needed.

Walnut shell extraction

The sample putted in a thimble in the Soxhlet extraction's conventional approach .Gradually filled with brand-new condensed extract (a name for the extraction solvent) from a distillation flask. The extracted analytics are transferred to the bulk liquid by the siphon, which suctions it from the thimble when the questioner reaches the overflow level and dumps it out again into a distillation flask.Until the extraction is finished completely, the operation is repeated. This performance Soxhlet creates hybrid technology that is both continuous and discontinuous.

Preparation JRSE and vitamin C concentration.

To evaluate the antioxidant activity, 1 mg of JRSE was weighed and diluted in 1 ml (DW).Than, concentrations were made (50, 40, 30, 20, 10, 5µg/ml) of JRSE in addition to vitamin C with the same concentrations and then added into wells in (96 wells of a microplate).

Preparation of(DPPH)

The antioxidant mechanism of the active compounds is established along with the use of the DPPH assay (McGowan, 1959).100 ml of 82% methanol were used to dissolve 3.2 milligram of DPPH to create the DPPH solution then it is kept in a dark place until use (Ul-Haq, 2012).

Antioxidant assay using (DPPH) radical scavenging

On a 96-well plate, 100 µl of DPPH solution was added and then 100 µl of supernatants (50,40,30,20,10,5 µg/mL) of ethanolic walnut shell extract and matched amounts of vitamin C were plated next to a control. The plate was incubated at room temperature for 30 minutes in the dark. Instead of testing the sample and the DPPH solution as a reference at the same time, a mixture of the two was investigated. Using a microplate reader to measure the absorbance at 570 nm, the IC50% (free radical inhibition rate) was determined using the equation below.

Inhibition% = [(A)control-(A)sample/(A)control)] × 100 % (ROSIDAH, 2018) (Hong, 2020).

RESULTS AND DISCUSSION

The Antioxidant Activity of (JRSE).

A scavenging assay for the antioxidant(DPPH) was used to measure the antioxidant activity of the JRSE. This test is renowned for providing accurate information on the antioxidant capacity of the examined substances (Neupane, 2021).

In this study, the antioxidant activity of walnut shell extract was determined using methanol solution of DPPH reagent and using vitamin C for comparison purpose, as in Table (4.1).

When the average antioxidant activity of walnuts was compared with vitamin C, walnut shell extract showed less antioxidant activity than vitamin C, where the IC50% was (21.97±0.1µg/ml) while the IC50% of vitamin C was equal to (18.35±0.1 µg/ml).

Table (4.1) DPPH scavenging activity (IC50%) of JRSE and vitamin C, sample Linear equation and coefficient of regression (R2).

sample	Linear equation	R^2	IC50% µg/ml

JRSE	**y = 1.2532x + 22.46**	**0.9833**	**21.97±0.1**
Vitamin C	**y = 1.1175x + 35.36**	**0.9593**	**18.35±0.1**

The results correspond with (Wang, 2020). They found that walnut shells are rich in phenolic compounds, which are characterized by their ability to scavenge DPPH radicals, as these phenols are a good natural source of antioxidants, which makes them an important source in the future. As (Jahanban-Esfahlan, 2019) explained, the shell and the husk of the walnut fruit are agricultural by-products, which are an excellent source of various phenolic compounds and other useful compounds that are produced when processing the fruit, and they have therapeutic potential or protective properties, as the results showed the strength of the walnut as a source of effective chemical protective components and natural antioxidants.

Cytotoxic activity of (JRSE) toward a certain cancer and normal cells in vitro.

Concentrations (31.25, 62.5, 125, 250, 500,1000) prepared from the plant extract were tested on two types of cancer cell lines, the first being prostate cancer cells (PC3), where the inhibition concentration of 50% of cells (IC50%) was 360.64±5.16 µg/ml. The second being lung cancer cells (A549), where the IC50% was 256.252±3.6µg/ml. The results showed that theIC50% was 430.03±1.90 µg/ml after incubating the cells for 24 hours and was measured using an ELISA platereader. Finally, the concentrations of the JRSE were tested on the normal cell line (L929) and the results showed that the value IC50% was 313.96±3.21µg/ml.Strong or high cytotoxic activity are defined by an IC50 value of less than 21µg/ml, moderate cytotoxic activity by an IC50 of between 21 to 200 µg/ml, and weakly cytotoxic activity by an IC50 of between 201 to 500 µg/ml; IC50 values more than 501 µg/ml are regarded as non-cytotoxic (Amaani, 2018). As a result, the extract has weakly cytotoxicity on the four cell lines listed above.

CONCLUSIONS

1. JRSE showed antioxidant activity lower than vitamin C.
2. JRSE showed cytotoxicity against normal and cancer cell lines.

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