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In vitro anticancer activities of *Withania coagulans* against HeLa, MCF-7, RD, RG2, and INS-1 cancer cells and phytochemical analysis

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ABSTRACT

Background: The Pakistani Salt Range has a rich floral diversity including *Withania coagulans* from the Solanaceae family.

Methods: The crude methanolic extracts of the root, leaf, leaf stalk, and fruit of this plant were screened for their cytotoxic activity against human (HeLa, MCF-7, RD) and rat (RG2 and INS-1) cancer cell lines at 20 μg/mL and compared to methotrexate. The IC_{50} values indicated that leaf stalk and fruit extracts exert an 80% or higher cytotoxic activity against all cell lines at 24 hours.

Results: The leaf stalk extract showed the highest cytotoxic efficacy against all tested cell lines, with IC_{50} values ranging from 0.96 ± 0.01 μg/mL to 4.73 ± 0.05 μg/mL followed by the fruit extract with IC_{50} values of 0.69 ± 0.01–6.69 ± 0.06 μg/mL after 48–72 hours incubation. The leaf stalk and seed extracts were analyzed for polyphenols and flavonoids using RP-HPLC. The total flavonoid content (TFC) was calculated for all tested samples, and the highest TFC was recorded for the root extract (394.34 ± 1.26 μg/g). The total phenolic content (TPC) was found in the seed extract (307.86 ± 9.42 μg/g) of *W. coagulans*. The highest contents of myricetin (358.46 ± 2.91 μg/g) were noted in the leaf extract, and highest quercetin was recorded in the seed extract (21.43 ± 0.13 μg/g). The highest gallic acid concentration (83.62 ± 0.71 μg/g) was recorded in leaf stalk extract and *p*-hydroxybenzoic acid in the seed extract (157.46 ± 1.43 μg/g).

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Conclusion: The present study gives a scientific insight and comparative analysis of various plant parts in this medicinally important plant species from the Salt Range of Pakistan against both human and rat cancer cells.

1. Introduction

Cancer caused over 8 million deaths worldwide in 2013; in 1990, it was the 3rd leading cause of death, so it advanced to the 2nd place in 2013. According to an estimate of the American Cancer Society (ACS), about 1,658,370 new cancer cases were diagnosed and 589,430 cancer deaths registered in the United States (US) alone during 2015. For some cancers, effective albeit expensive therapies are available, whereas no effective therapies are available for others.

Plants are an important source of natural products providing the raw material for diverse pharmaceutical and therapeutic applications due to the presence of phytochemicals commonly known as secondary metabolites. A large number of metabolites are utilized against various diseases including cancer and other cellular disorders.[1,2] The medicinal plants and their bioactive constituents have been extensively used as therapeutics against various cancer types, and a vast number of medicinal plants belong to the Solanaceae family.[3,4]

Medicinal plants are important sources for the treatment of noxious diseases like cancer in developing countries like Pakistan. Plants are used due to their cost-effectiveness, availability, and low toxicities.[5] Scientists now need to focus primarily on the scientific validation of the plant species that are used in folk medicines based on ethnobotanical surveys. Once promising plant species are identified, identification of the bioactive substance and their therapeutic effects are subsequent tasks.[5]

Pakistan has a diverse flora of medicinal plants starting from the deserts of Sind, passing through the plane of Punjab to the Hilly areas of northern Pakistan. The salt range of Pakistan is also very rich in its medicinal plants, traditionally used by local herbalists (*Hakeems*) to treat various noxious diseases including cancer.[6] One important plant family of the region is Solanaceae, and *Withania coagulans* is one important regional endemic, edible, and medicinal plant of this family. The plant is ethnobotanically reported in cancer treatment by local practitioners (*Hakeems*) as well as other noxious diseases for centuries; however, scientific authentication of its use has yet not been established. Concerning phytochemical isolation and cytotoxic activity, no study has been reported to date on *W. coagulans* from Pakistan. This plant is commonly known as Indian Rennet, vegetable rennet (English), Paneer Dodi/Jangli Paneer (Hindi & Punjabi), and Ning gu shui qie (Chinese). It has been reported to possess a variety of ethnomedicinal uses, and its extracts have shown potential activities, in particular, anticancer activities, wound healing, immune modulating, as well as antihyperglycemic and hypolipidemic activities.[7]

A plant's secondary metabolites are synthesized in response to stress conditions, and salt stress is one of the inducing factors. The plant phenolics have a major role to combat oxidative stresses to prevent cellular damages which can lead to DNA damages and abnormal cell division. In this context, the plant phenolics can be used as a cancer chemopreventive or cancer chemotherapeutics agents. Keeping in view, the present study was conducted to validate cytotoxicity effects of this plant's extracts and its fractions in comparison to its phenolic contents against MCF-7 (breast), HeLa (cervix), RG2 (brain), RD (rhabdomyosarcoma), and INS-1 (pancreas) cancer cell lines, following the guidelines and recommendation of the United States NCI plant screening program and analysis of their phytochemicals.

2. Materials and methods

2.1. Chemicals

Methanol HPLC grade, 99.9% and n-hexane, 95% were purchased from Sigma–Aldrich, USA; dimethyl sulfoxide (DMSO) was obtained from Fisher BioReagents, Fair Lawn, NJ.

2.2. Collection of plant material

W. coagulans plant material was collected during a field survey in April 2013 from different growing localities in the Salt Range (Fig. 1) Punjab, Pakistan (32.2416°N, 72.0237°E, and 867 m elevation). One set of specimens was prepared and identified in the Taxonomy Lab., Department of Botany, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, following the methodology of Qureshi et al.[8] The voucher specimen (Specimen No. MAQ-313(01)-2013) was deposited in the Department Herbarium of Botany, PMAS-Arid Agriculture University Rawalpindi.

2.3. Preparation of crude extract

The plant material (fruit, leaf, leaf stalk, and root) was washed, cut into pieces, dried in the shade, and pulverized using a grinder. The fine plant powder (1 kg of each) was soaked in methanol for 5–10 days to extract soluble compounds with the remaining material removed by filter paper. The first crude methanolic extract (CME) was obtained by concentrating the filtrate under reduced pressure in a rotary evaporator and further dried in a vacuum oven at 40°C. The CME was stored at 4°C in the dark until further use.[9,10]

2.4. Preparation of stock solution of crude extract

The dried plant extracts of fruit, leaf, leaf stalk, and root were weighed using a standard analytical balance (OHAUS, model

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