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Research Article

Study of Anticancer and Antibacterial Activities of *Podophyllum Hexandrum* as Natural Curatives

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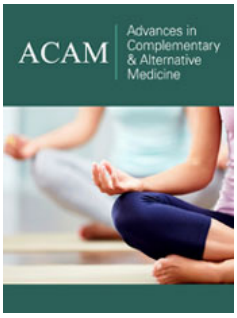

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Abstract

The utilization of medicinal plants to treat infectious disease is a common practice in developing countries worldwide. The present study was aimed at evaluating the crude extracts of *Podophyllum hexandrum* with different chemicals for anticancer and antibacterial potential. The results revealed that the methanol fractions of P. hexandrum showed significant cytotoxic potential against MCF-7 with lowest %IC50 of 41±1.79 than other extracts used in this study. Furthermore, crude extracts were also analyzed for antibacterial potential against six multi drug resistant (MDR) pathogens by disc diffusion method. The n-hexane extract showed good inhibitory effect against all the tested strains except *S. typhi*. Its highest activity was observed against *E. coli* with zone of inhibition of 22±1.25 (MIC=25μg/mL). Methanol and ethanol extract also depicted inhibitory potential for most of the tested bacterial strains. In summary, *P. hexandrum* extracts possess antibacterial and anticancer activities, suggested to be a strong pharmaceutical agent.

Keywords: Mcf-7; *Podophyllum hexandrum*; Antibacterial; Medicinal plants; Cytotoxic

Introduction

Antimicrobial obstruction (AMO) is a genuine wellbeing concern that has added to increases in horribleness, mortality, and human services expanse. Strains from different clinical and natural sources, essential cells for medicate danger screening studies and media and reagents to help cell growth are perfect for the advancement, check, and assessment of quick discovery strategies, imaginative remedial procedures, novel anti-toxins, and refreshed sterility conventions. Because of enzymatic inactivation for example aminoglycoside altering proteins, and β-lactamases. The extract with different compounds from therapeutic plants have been tried and demonstrated the viability of conventional herbs against microorganisms; thus, plants are one of the bedrocks for present day drug to accomplish new standards [1]. Accordingly, restorative plants can offer a riches for their organic exercises, for example, antimicrobial, cancer prevention agents, antimalarial, and anticancer exercises.

Cancer is a deadly sickness in entire world with high pace of death cause and psychological state. As per the count of 2012, 14.1 million new instances of disease appeared in worldwide because of which 8.2 million passing's happened [2]. Modification of base and other hereditary changes happen because of increment in level of receptive oxygen species. These species are framed from extra cell and intracellular sources. These species consolidate with deoxyribonucleic corrosive and cell proteins [3]. These redesigning causes an enormous change in the ordinary procedure of apoptosis which causes the undesirable and boundless cell division and furthermore prompts tumor development [4]. Organized researches depicted that development and progression of cancer results from intense multiplication of abnormal cells. During the process of cell division of cancer cell, many dynamic aberrations emerge in genome and accumulation of such genetic abnormalities direct the transfer of normal cells to tumor cancer cell [5].

Natural products are considered generally as the rich wellspring of phytochemicals with different bio-structures and strong bioactivities against various ailments including malignant growth and irresistible maladies [6]. In spite of the fact that the chemotherapeutic medications are the great alternative to cure from malignant growth, however they are not

without certain downsides, for example, extreme symptoms and medication obstruction and so forth. Thusly, investigating novel plant determined bioactive operators having hostile to malignant growth and antimicrobial potential would add to deal with the medication opposition and poisonous quality.

Podophyllum hexandrum Roylesyn P. emodi Wall. ex Hook. f. and Thoms is accepted to be originated from Himalayan area. It is a significant restorative plant known for important medication podophylotoxin which is successful against different sicknesses including moles and tumors development of skin and have various properties, for example, laxative, purgative, cholagogue and emetic [7]. *P. hexandrum* has been portrayed as celestial medication in the Indian conventional arrangement of medication, the Ayurveda and has additionally been utilized in customary Chinese framework. The plant additionally got colossal potential in Unani, and Siddha arrangement of medication for the treatment of different ailments [8].

Materials and Methods

Plant collection and preparation of concentrates

The plant samples of *Podophyllum hexandrum* were gathered from Khyber Pakhtunkhwa (Pakistan) in the long stretch of August and September. The material of gathered plant was exhaustively washed with running tap water and dried under shade at room temperature. The dried example of plant was ground to powder structure and concentrates were stored by keeping in a firmly shut holder. The leaf unrefined concentrates of *P. hexandrum* were set up in five solvents with various extremity by drenching 25g plant material in 250ml of methanol (PM), ethanol (PE), benzene (PB), chloroform (PC) and n-hexane (PH) for one week with unpredictable shaking. The blends were sifted through Whatman channel paper No. 1 and filtrate was packed in a rotational evaporator (Rotavapor R-200 Buchi, Switzerland) at a temperature of 45 °C. The buildup was splashed again in particular solvents and procedure rehashed multiple times. Each of the samples that were extricated kept at - 20 °C until further use.

Antibacterial Assay

The concentrates of plants were examined for antibacterial potential for six clinically refined MDR bacterial strains *viz Klebsiella pneumoniae*, Methicillin-resistant *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Serratia marcescens* and *Pseudomonas aeruginosa* picked up from IBGE (Institute of Biomedical and Genetic Engineering) Islamabad Pakistan while Enterobacter aerogenes ATCC# 13048 and *Staphylococcus aureus* ATCC# 6538 were utilized as reference delicate strains. Disc diffusion strategy [9] was utilized to asses antibacterial strength. For negative control, DMSO was utilized. Minimum inhibitory concentrations (MICs) were noted only for those concentrates that had a restraint zone comparable to or in excess of 10mm by micro broth dilution methodology with minor changes [10].

Cytotoxicity Analysis

Breast cancer cell line (MCF-7) was cultured in 96 well plate at a density of 1×105 per ml by incubating at 37 °C for 24h under 5% CO_2. Then the cell medium was inoculated by different concentrations of plants extract and incubated for 48 hours. After 48 hours of treatment, 50% inhibitory concentrations (IC50) were determined by MTT assay [11].

Results

The MDR clinical bacterial strains were investigated for antibiotic sensitivity and resistance against commonly available antibiotics [9]. The antimicrobial activity of *P. hexandrum* extracts prepared in five different solvents was assessed by measuring the diameter of inhibition zones using disc diffusion method (Table 1) against selected MDR isolates i.e; *K. pneumoniae, E. coli, S. typhi, S. marcescens, P. aeruginosa* and MRSA. *Staphylococcus aureus* ATCC# 6538 and *Enterobacter aerogenes* ATCC# 13048 were also used as reference sensitive strains.

Table 1: Antibacterial activity of *P. hexandrum* extracts against MDR bacterial strains and ATCC reference strains.

Extract solvents	*K. pneumoniae*		*P. aeruginosa*		*S. typhi*		*S. marcescens*		*E. coli*		MRSA		ATCC *E. aerogenes*		ATCC *S. aureus*	
	ZOI (mm) ±SD	MIC(μg/mL)	ZOI (mm) ±SD	MIC(μg/mL)	ZOI (mm) ±SD	MIC (μg/mL)	ZOI (mm) ±SD	MIC (μg/mL)	ZOI±SD	MIC (μg/mL)	ZOI (mm) ±SD	MIC (μg/mL)	ZOI(mm) ±SD	MIC (μg/mL)	ZOI (mm) ±SD	MIC (μg/mL)
PM	13±1.33	75	7±0.75	----	----	----	18± 0.85	50	8± 0.75	----	11±0.55	----	15±1.22	75	17± 0.55	50
PE	17±1.25	50-	----	----	11±1.00	75	12±1.25	75	11±0.89	----	15±0.89	75	14 ±1.25	75	18± 1.25	50
PB	-----	75	9±0.85	----	-----	----	----	----	9±0.50	----	15± 1.45	75	10±0.75	----	11±0.75	----
PC	8±0.75	----	----	----	15± 1.00	50	----	----	-----	-----	14±0.75	75	14±1.20	75	16± 1.00	50
PH	18±1.50	50	12±1.00	----	-----	----	10±0.50	----	22±1.25	25	21±0.75	25	19±1.00	50	17± 1.20	50
DMSO	---	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----

Experimental data is articulated as mean ±SD (n=3)

----: no zone of inhibition

P. hexandrum plant concentrates utilized against the pathogenic strains have demonstrated differed level of antimicrobial action. In this investigation, the concentrates producing a development inhibitory zone ≥10mm in were viewed as dynamic and were additionally evaluated for MIC assurance through stock microdilution strategy. MIC is characterized as the most reduced centralization of each example that avoided this change and exhibited total restraint of microbial development. Methanolic concentrates of *P. hexandrum* demonstrated most extreme Zone of inhibition (ZOI) against *S. marcescens* with 18±0.85 (MIC: 50µg/ml) trailed by ATCC *S. aureus* with 17±0.55 (MIC: 50µg/ml) while its ethanolic concentrates demonstrated most extreme ZOI against ATCC *S. aureus* 18±1.25 (MIC: 50µg/ml) trailed by *K. pneumoniae* 17±1.25 (MIC: 50µg/ml). Likewise, benzene extricates against MRSA 15±1.45 (MIC: 75µg/ml) and chloroform extract against ATCC *S. aureus* 16± .00 (MIC: 50µg/ml) followed by *S. typhi* 15±1.00 (MIC= 50µg/ml) indicated most extreme Zone of hindrance. Hexane concentrates of *P. hexandrum* demonstrated greatest Zone of inhibition (ZOI) against *E. coli* 22±1.25 (MIC=25µg/ml) followed by MRSA 21±0.75 (MIC= 25µg/ml).

Extracts of *P. hexandrum* were analyzed against Estrogen Receptor positive breast cancer, MCF-7 cell line (Figure 1). The cells viability was determined after 48h treatment with indicated concentrations of each extract. Almost all the extracts inhibited the proliferation of MCF-7 cells using various concentrations, but strongest effect was exerted by methanol extract of *P. hexandrum*. Both extracts (methanol and ethanol) were more effective against breast cancer cells as compared to other extracts in this study. This inhibitory effect was enhanced by increasing concentrations. Methanolic extracts of *P. hexandrum* showed highest cytotoxicity against MCF-7 with lowest %IC50 of 41±1.79µg/ml than other extracts followed by ethanol extract exhibiting %IC50 of 58±2.25µg/ml.

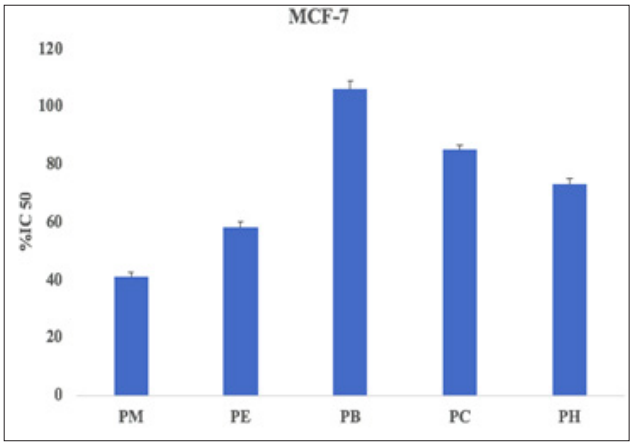

Figure 1: Cytotoxicity of P. hexandrum extracts against MCF-7 cell line. Data was articulated as mean (IC50)±SEM (n=3) of three independent experiment.

Discussion

The underuse, overuse, and misuse of antibiotics by humans are the selective pressure, which eventually lead to the development of antibiotic resistance in microbes [12]. MDR microorganisms can survive the treatment with antimicrobial drugs, thereby standard treatments become ineffective and infections persist, increasing the risk of spread to others. In general, MDR microbes are resistant to three or more antibiotics [13]. The emergence of multidrug resistant pathogenic bacteria has created medical challenges for their treatment. *In vitro* pharmacological screening of traditionally used medicinal plants offers an incredible opportunity to explore and investigate capacity of novel therapeutic agents from a wide range of plant to certify claims related to their safety and efficacy.

Plant used in present study is medicinally important. *P. hexandrum* is a source of various biologically important metabolites and possess antioxidant, anti-inflammatory, antifungal, cytotoxic and radioprotection activity [14,15]. It is an important medicinal plant known for valuable drug podophylotoxin which is effective against various diseases including warts and tumors growth of skin and possess different properties such as purgative, laxative, cholagogue and emetic [7].

Bioactive compounds are commonly extracted by organic solvents such as ethanol, acetone, and methanol as most of the polar compounds are easily eluted by these solvents which is bioactive responsible for their activity. In the present study methanol extract of the *P. hexandrum* showed activity against all the human pathogens except the *S. typhi*. The peak value of zone of inhibition was shown against the *S. marcescens* i.e. 17±0.85mm. Meanwhile in ethanol extract, the highest value of resistance was shown for ATCC *S. aureus*. Moreover *S. typhi* did not show any zone of inhibition in benzene and hexane extracts while the highest zone of inhibition was shown for MRSA and *K. pneumoniae* respectively in these extracts.

It is well known that cancer is the second major cause of death in the worldwide. Thus, leads to search new drugs of natural products that could contribute to overcome other treatment strategies of cancer like radiation therapy [16]. In the present study, in addition to the drug resistance, the anticancer effects of *P. hexandrum* were

focused. Methanol extract of the plant *P. hexandrum* depicted the least %IC50 values for MCF-7 cell line i.e. 41±1.79μg/ml. This showed that the methanol extracts of the plant could have potent anticancer molecules. Isolation of bioactive molecules from *P. hexandrum* will prove to be helpful in pre-clinical trials to explore true potential of these extracts/bioactive molecules against different cancers.

Conclusion

Natural dynamic compound from plant material is for the most part reliant on the sort of the solvent utilized in the extraction methodology. In the present examination, *P. hexandrum* extracts in different solvents had antibacterial and anticancer activities; this may be useful in forestalling or easing back the advancement of malignant growth and against pathogenic bacterial strains. In this way, they could be potential contender for pharmaceutical medications ventures. Further examination on the disengagement and recognizable proof of anticancer and antibacterial part in the plant may prompt concoction elements with potential for clinical use.

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