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Sterculia diversifolia bears anti-cancer and immunomodulatory activities

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Abstract

The present study was aimed to evaluate the methanolic extract and subsequent solvents soluble fractions of *Sterculia diversifolia* bark for cytotoxic, anti-cancer and immunomodulatory activities. Phytochemical investigation confirmed the presence of alkaloids, flavonoids, saponins, glycosides, etc. In the cytotoxic activity, *n*-hexane showed potent activity (LD_{50}: 7.0 µg/mL) followed by dichloromethane fraction (LD_{50}: 16.2 µg/mL). In the anti-cancer activity, dichloromethane fraction showed potent activity (IC_{50}: 5.9 µg/mL) followed by ethyl acetate fraction (IC_{50}: 9.5 µg/mL). While in the immunomodulatory assay, ethyl acetate fraction showed a very significant activity (IC_{50}: 21.0 µg/mL) followed by diclorometh ane and *n*-butanol fractions (IC_{50}: 25.0 and 25.3 µg/mL respectively). Hence, it is clear that *S. diversifolia* has anti-cancer and immunomodulatory agents.

Introduction

A variety of pharmacologically active compounds such as quercetin, apigenin and scopolin have been isolated from the leaves of *Sterculia foetida* (Rani et al., 2010). Sterculinine-I (Wang et al., 2003), sterculinine-II (Wang et al., 2003) and soyacerebroside-I (Shetty et al., 2014; Wang et al., 2013) were isolated from the seeds of *S. lychnophora*. Similarly, epicatechin, procyanidin B2 and C_4-C_8 dimers of epicatechin were isolated from the stem bark of *S. tragacantha* (Orisakeye and Olugbade, 2014). A variety of pharmacological activities such as anti-oxidant (Prakash and Kaviarasan, 2012), antimicrobial (Shivakumar and Vidyasagar, 2014), cytotoxic (Prakash and Kaviarasan, 2012), CNS depressant (Mujumdar et al., 2000), anti-inflammatory activity (Prakash and Kaviarasan, 2012), anticonvulsant activity (Raja et al., 2014), antifungal, genotoxic (Van den et al., 2008), anti-diabetic (Hossain et al., 2012), anthelmintic (Alam et al., 2012), and analgesic activity (Hossain et al., 2014) have been reported from the members of Sterculiaceae.

S. diversifolia is a medium size tree. The plant bears laxative, anti-bacterial, antifungal and anti-oxidant activities. Previously few fatty acids have been reported from *S. diversifolia* (Salem et al., 2014). The current study is aimed to investigate the methanolic extract of *S. diversifolia* and its subsequent solvents soluble fractions for its prospective cytotoxic, anti-cancer, and immunomodulatory activities.

Materials and Methods

Plant material

Plant material was collected from the botanical garden of Pakistan Forest Institute, University of Peshawar, Pakistan in September, 2014. Plant material was identified by Mr. Ghulam Jelani, a taxonomist at the Department of Botany, University of Peshawar. A specimen

was deposited at the herbarium of the University of Peshawar under reference No. Bot.20098 (PUP).

Extraction and fractionation

The stem bark of the plant (17 kg) was air dried in the shade at ambient temperature and then crushed to powder. Dry powder was subjected to maceration using methanol for 14 days (2 × 7 days) and then filtered using Whatman No. 1 filter paper. The extract was concentrated using rotary evaporator under reduced pressure at 40°C (Ullah et al., 2016a). Methanolic extract (950 g) was mixed with 2.5 L distilled water and soaked for 24 hours, then extracted successively with *n*-hexane (3 × 2.5 L), dichloromethane (3 × 2.5 L), ethyl acetate (3 × 2.5 L), and *n*-butanol (3 × 2.5 L) to obtain their respective soluble fractions. The remaining was considered as water soluble fraction i.e. aqueous fraction.

Preliminary phytochemical screening

Methanol extract was preliminary evaluated for qualitative phytochemical analysis using the standard protocols (Kayani et al., 2007; Ullah et al., 2016b).

Brine shrimp lethality

To determine the cytotoxic potential of the methanol extract and fraction, brine shrimp lethality bioassay was performed. In this method, artificial sea water was prepared by dissolving 38 g of sea salt in double distilled water, pH 7.4 and then filtered (Meyer et al., 1982). Brine shrimp larvae were produced by placing the sea water in a small tank and then adding brine shrimp eggs and allowing it to stand for 24 hours at 25° C. During all this process, this tank was covered with aluminum foil. Stock solutions of the test sample (methanol extract and fractions) were prepared by dissolving 20 mg of the sample in 2 mL of chloroform. Then 1000, 100 and 10 µg per mL concentration of the test samples were obtained by transferring 500, 50 and 5 µL of the stock solution into vial respectively. Then three replicates were prepared for each concentration making a total of nine vials. The solvent was allowed to evaporate. Then 10 larvae were placed in each vial and volume was made to 5 mL by adding sea water. Two vials were supplemented with solvent and reference cytotoxic drug served as negative and positive control respectively.

The standard reference cytotoxic drug used was etoposide (LD_{50} = 7.5 µg/mL). These entire vials were incubated for 24 hours at 25-27°C. After incubation time, the number of survivals was counted. To determine the LD_{50}, Finney computer program was used (Alves et al., 2000).

Anti-cancer (PC3) activity

Anti-cancer activity was recorded in 96-well microplate by MTT assay. Human prostate cancer cells (PC-3) were cultured in DMEM (Dulbecco's Modified Eagle's Medium), along with 5% of fetal bovine serum, 100 IU/mL of penicillin and 100 µg/mL of streptomycin in 75 cm^2 flasks and kept in 5% CO_2 incubator at 37°C. Exponentially growing cells were harvested, counted with hemocytometer and diluted using the medium. Cell culture with the concentration of 1 x 10^5 cells/mL was prepared and introduced (100 µL/well) into 96-well plates. After incubation, the medium was removed and 200 µL of fresh medium was added with concentrations of test samples (1-30 µM). After 48 hours, 200 µL MTT (0.5 mg/mL) was added to each well and incubated further for 4 hours. 100 µL of DMSO was added to each well. The extent of MTT reduction was calculated by measuring the absorbance at 570 nm, using a microplate reader. The cytotoxicity was measured as concentration causing 50% growth inhibition (IC_{50}) for PC-3 cells. The percent inhibition was determined by using the following formula:

%Cell inhibition =

1 – (Absorbance of sample/absorbance of control) x 100

Immunomodulatory assay

Luminol-enhanced chemiluminescence assay was performed using standard protocol (Mesaik et al., 2009). Briefly, whole blood (diluted 1:200) neutrophils (1 × 10^7) and polymorphonuclear leukocytes (1 × 10^6) were suspended in Hank's balance salt solution (HBSS) with calcium and magnesium and incubated with 50 uL of test compounds concentrations (1.6 to 50 µg/mL) for 30 min. To each well, 50 µL (20 mg/mL) zymosan (Sigma Chemical Co. USA), followed by the addition of 50 µL (7 × 10s M) luminol (G-9382 Sigma Chemical Co.) and then HBSS were added to adjust the final volume to 0.2 mL. HBSS was used as a control. Chemiluminescence's peaks were recorded with a luminometer (Luminoskan RS Lab, Finland).

Results

The methanolic extract of *S. diversifolia* was screened for the presence of various phytochemicals (Table I). From the results, it was confirmed the presence of alkaloids, carbohydrates, saponins, sterols, steroids, glycosides, flavonoids, phenol's, tannins, phalbotannins, terpenoides and vitamin C.

Brine shrimp lethality (cytotoxicity)

The results for methanolic extract of *S. diversifolia* and various solvents soluble fractions against brine shrimp lethality assay is presented in Table II. A typical concentration dependent cytotoxic effect was observed. *n*-Hexane fraction showed a maximum activity with LD_{50} value of 7.0 µg/mL followed by dichloromethane fraction with an LD_{50} value of 16.2 µg/mL. The rest of the samples showed a mild to moderate cytotoxic behavior. LD_{50} value was 7.5 µg/mL for etoposide (standard).

Table I

Phytochemical screenings of *Sterculia diversifolia*

Chemical class	Test	Observation
Carbohydrates	Molisch's test	Positive
	Benedict's test	Positive
	Soluble starch test	Positive
	Iodine test	Positive
	Salivin off's test	Negative
	Barfoed's test	Positive
	Osazone test	Positive
Alkaloids	Hager's reagent	Positive
	Wagner's reagent	Positive
Saponins	Vigorous shaking	Positive
Phenol's	Phenol's	Positive
Flavonoids	Ferric chloride test	Positive
	Lead acetate test	Positive
	Sodium hydroxide test	Positive
Sterols	Sterols	Positive
Steroids	Steroids	Positive
Glycosides	Keller Killani test	Positive
	Bromine water test	Positive
	Anthraquinones test	Negative
Coumarin's	Coumarin's	Negative
Terpenoids	Terpenoids	Positive
Tannins	Tannins	Positive
Phalobotannins	Phalobotannins	Positive
Amino acids	Ninhydrin test	Negative
	Lead acetate test	Negative
Proteins	Biuret test	Negative
	Saturation test	Negative
Vitamin C	Vitamin C	Positive

Anti-cancer activity (PC 3 cell lines)

The methanolic extract of *S. diversifolia* and various fractions were investigated for anti-cancer activity against PC-3 cell lines (Table II). A significant anti-cancer potential was observed in case of dichloromethane fraction with IC_{50} values of 5.9 ± 0.3 µg/mL, followed by ethyl acetate (9.5 ± 0.4 µg/mL), and methanolic extract of *S. diversifolia* (16.4 ± 0.3 µg/mL). Rest of the samples were fairly inactive in the anti-cancer assay. IC_{50} value for the doxorubicin (standard) was 2.8 µg/mL.

Immunomodulatory activity

The methanolic extract of *S. diversifolia* and various fractions were evaluated for immunomodulatory activity (oxidative burst assay) (Table II). The inhibition of reactive oxygen species (ROS) was observed by calculating their IC_{50} values. The maximum ROS inhibition was observed for ethyl acetate fraction, followed by dichloromethane and *n*-butanol fractions with IC_{50} values of 21.0, 25.0, and 25.3 µg/mL respectively. A moderate activity was observed in case of ethyl acetate fraction with IC_{50} value of 95.0 µg/mL. While the metha-nolic extract of *S. diversifolia*, *n*-hexane and aqueous fractions didn't showed any significant activity. The IC_{50} value for ibuprofen (standard) was 11.2 ± 1.9 µg/mL.

Discussion

Plants produces pharmacologically active compounds of different chemical classes which are deposited in their specific parts. Phytohemical tests revealed the presence of alkaloids, carbohydrates, saponins, sterols, steroids, glycosides, flavonoids, phenol's, tannins, terpenoides and vitamin C, while negative result for coumarins, amino acids and proteins in methanolic extract of *S. diversifolia*. Presence of the compounds of the chemical classes such as alkaloids, steroids, couma-

Table II

Brine shrimp lethality assay, anti-cancer activity and immunomodulatory assay of *Sterculia diversifolia*

Sample	Brime shrimp lethality assay	Anti-cancer activity	Immunomodulatory assay	
	LD_{50} (µg/mL)	IC_{50} (µg/mL)*	%Inhabition/ stimulation (25 µg/mL)	IC_{50} (µg/mL)*
Methanol	169.5	16.4 ± 0.3	44.0	-
n-Hexane	7.0	19.3± 0.4	37.2	-
Dichloromethane	16.2	5.97 ± 0.3	-	25 ± 5.0
Ethyl acetate	184.4	9.5 ± 0.4	-	21 ± 2.3
n-Butanol	127.4	> 30	-	25.3 ± 2.9
Aqueous	166.8	> 30	22.1	-
Etoposide (Standard)	7.5	-	-	-
Doxorubicin (Standard)		2.8 ± 0.1	-	-

*Data are mean ± SD

rins, tannis, glycosides and tannins validates its anti-cancer and immunomodulatory potentials (Alam et al., 2016).

A variety of plants, their extracts, and isolated compounds have proved their use for the treatment of various cancers. Sterculiaceae family is very familiar for containing plants bearing anti-cancer potential. Brine shrimp lethality activity is an easy, convenient, and reproducible bioassay to assess the samples for their possible cytotoxic potential. Compared to positive control (etoposide LD_{50}: 7.5 µg/mL), all the tested samples showed good brine shrimp lethality activity. Samples bearing less than 250 µg/mL, LD_{50} were considered significantly active (Rieser et al., 1996). Potent cytotoxic activity indicated the samples to be further assessed for further investigation in various anti -cancer models (Meyer et al., 1982; Apu et al., 2010).

Keeping in mind the cytotoxic activity, the anti-cancer activity was performed to clarify the anti-cancer potential of methanolic extract of *S. diversifolia* and subsequent solvents soluble fractions. The results of anti-cancer activity (PC3 cell lines inhibition) showed that this plant can be recommended in the management of cancer (Alam et al., 2016). From the result it is clear that all those samples effective in cytotoxic activity were fairly active in the anti-cancer activity as well indicting the presence of a number of chemical constituents responsible for its anti-cancer potential. The main objective of cancer management with chemotherapy (anti-cancer drugs) is to kill/inhibit the neoplastic cells. There are different cells which are naturally present in the human body. These cells are part of the immune system including natural killers, cytotoxic cells and lymphokine activated cells, which are responsible to destroy abnormal and damaged cells (Alam et al., 2016). Any agent which has got the property of cytotoxicity can be used in various pathological conditions (inflammation, AIDS, infection and cancer) (Su et al., 2009).

The ethyl acetate fraction showed good inhibitory effect against ROS followed by dichloromethane and *n*-butanol, which is clear from their IC_{50} values while the methanol extract, *n*-hexane and aqueous fractions possess no activity. From these results we conclude that the active chemical agent responsible for inhibition of ROS may be present in the ethyl acetate, dichloromethane and *n*-butanol fraction. All organisms (aerobic) produce ROS, which can easily react with different bio molecules like proteins, lipoproteins, lipids and DNA (Ullah et al., 2016c). ROS play an important role in the development of cancer. Generation of ROS is due to drugs, pesticide and other pollutants derived from tobacco cause destruction of membrane lipids, proteins and DNA (Ciolino and Levine, 1997). Various diseases including diabetes, cancer, inflammation and arthritis are associated with ROS. In living system there is a protective phenomenon which provides protection against these ROS. Anti-oxidant agents are found naturally in tissues, they work as anti-aging substances. They diminish the oxidative reaction by free radical scavenging or by chelate formation (Kourounakis et al., 1999).

Conclusion

S. diversifolia showed a very potent cytotoxic and anti-cancer behavior against brine shrimp and PC-3 cell lines.

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Conflict of Interest

Authors declare no conflicts of interest

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