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Research Article
Effect of *Thevetia peruviana* Seeds Extract for Microbial Pathogens and Cancer Control

[1]T.M. Abdelghany, [2,3]Reham Yahya, [4]Marwah M. Bakri, [5]Magdah Ganash, [6]Basma H. Amin and [7,8]Husam Qanash

[1]Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Cairo, Egypt
[2]Medical Microbiology, College of Science and Health Professions, King Saud Bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia
[3]King Abdullah International Medical Research Centre, Riyadh, Saudi Arabia
[4]University College, Al-Ardah, Jazan University, Jazan, Saudi Arabia
[5]Department of Biology, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia
[6]The Regional Centre for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt
[7]Medical Laboratory Science, College of Applied Medical Sciences, University of Hail, Hail, Saudi Arabia
[8]Molecular Diagnostics and Personalized Therapeutics Unit, University of Hail, Hail, Saudi Arabia

Abstract

Background and Objective: The world is still in urgent need of natural compounds to solve many problems related to human health, especially from plants. Therefore the current study aims to assess the pharmacological activity of *Thevetia peruviana* extract. **Materials and Methods:** Anticancer against prostate cancer cell lines, antioxidant by Ferric Reducing Antioxidant Power (FRAP) assay and antimicrobial by disc diffusion method activities of *T. peruviana* (family Apocynaceae) Seed Kernel (SK) and Seed Pericarp (SP) extract were evaluated. Analysis of SK and SP phytoconstituents by GC-MS was performed. **Results:** Cytotoxic activity of SK and SP extracts were investigated and showed strong cytotoxicity with critical effects on the proliferation at IC_{50} values 0.05 and 35 μg mL^{-1}, respectively with remarkable morphological changes. The SK and SP extracts displayed antioxidant activity with 55.55 and 18.19 μm mg^{-1}, respectively. SK extract showed the highest antibacterial and anti-yeast activity than SP extract besides the appearance of changes in the ultrastructures of *Klebsiella pneumoniae*, *Bacillus subtilis* and *Candida albicans* exposed to SK extract. **Conclusion:** The obtained finding suggested that the methanolic extracts of SK and SP are rich in phytoconstituents and considered a promising new source particularly SK with biological activities that contribute to human diseases treatment.

Key words: Antitumor, antimicrobial, antioxidant, seed extract, *Thevetia peruviana*, seed pericarp, prostate cancer cell

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Corresponding Author: T.M. Abdelghany, Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Cairo, Egypt

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Many problems including detrimental effects and drug resistance regarding synthetic drugs utilization were documented[1]. Therefore, alternative and complementary treatment is emerging as a probable solution. Bioresources such as plants as well as their extracts represent excellent and reliable sources for the treatment of various and different infections such as microbial and cancer. Not only for infections treatment but for enhancing the immune system[2]. Much medicinal commerce searches for the traditional plants to discover new safe drugs derived from their natural constituents such as phenolics, alkaloids, terpenes and steroids[3-7].

Thevetia peruviana commonly called Yellow Oleander represent one of the members of the Apocynaceae family, presently it is distributed and broadly grown in numerous countries for ornamental goals. According to Ibiyemi[8], more than 400-800 globular, slightly fleshy fruits were recorded for *T. peruviana* plants depending on rainfall and plant age. Although some studies mentioned the toxicity of *T. peruviana* to the greatest of animals due to the existence of cardiac glycosides[9], other studies recognized its therapeutic benefits. All parts of *T. peruviana* contain a white sap that involves a compound known as Thevetin which plays a critical role as a stimulator for the heart but its poisonous feature was identified[9]. Surprising, some people in Batticaloa district Sri Lanka relied on Yellow Oleander Seed Poisoning (YOSP) that considered one of the responsible for commit suicide, therefore Madhura *et al.*[10] attempt to discover a link between the YOSP and fruiting season of *T. peruviana*. Traditionally as mentioned earlier[11], leaves of *T. peruviana* are well recognized as an abortifacient.

In the current era, according to medical reports, cancer has become the next main cause of fatality after heart attacks for all advanced and un-developed populations of the world. Many parts of the body do not escape cancer. Some of them greatly affects the body while others can be controlled. Scientists have shown that the rate of injury and death due to prostate cancer varies from one geographical region to another and also varies according to ethnic populations[12], currently the lowest rate in Asia and the highest rate in North America. Numerous etiological factors contribute to prostate cancer developing such as smoking, inflammation, consumption of high-caloric food and aging[13].

The anticancer potential of *T. peruviana* fruits was reported against numerous cancer cells. Remarkable effects were observed on the motility, proliferation and adhesion of breast and colorectal cancer cells, besides apoptosis stimulation in lung and prostate cancer cell lines[14-17], on the other hand, mild effects were recorded on non-tumorigenic cell lines. Although Dixit *et al.*[18] mentioned the presence of good antibacterial activity of *T. peruviana* fruits and flowers, he cautioned against using it due to its toxicity. Other biological activities including bactericidal and fungicidal properties particularly against common human dermatophytes pathogens were associated with *T. peruviana*[14]. The antimicrobial activities were interpreted by the presence of phenolic compounds in the extract of *T. peruviana*[19]. The inhibitory activity of *T. peruviana* was reported against variable bacteria but the potential of bacterial inhibition is dependent on the method and solvent used of extraction[20].

Different assays confirmed the antioxidant activities of *T. peruviana* extract[16]. Numerous phytochemicals were detected in *T. peruviana* leaves extract and its antioxidant potential was reported recently[21].

The current paper highlights the detection of *T. peruviana* constituents and biological activities. Research for natural quality biological material, as well as for innovative bioactive molecules was required for days to prevent the progress of various diseases, therefore the current study aims to assess the biological activity of Seed Kernel (SK) and Seed Pericarp (SP) extracts of *T. peruviana* seeds.

MATERIALS AND METHODS

Duration time of study: The study was carried out at the Department of Botany and Microbiology, Faculty of Science Al- Azhar University, Cairo, Egypt; Biology Department, Faculty of Science, King Abdulaziz University, Saudi Arabia; University College, Al-Ardah, Jazan University, Jazan, Saudi Arabia and Medical Laboratory Science, College of Applied Medical Sciences, University of Hail, Hail, Saudi Arabia from August 2020 and continue till March, 2021.

Plant sample collection and extraction process: A cultivated plant in the farm of T.M. Abdelghany (Corresponding author) placed in Monufia Governorate, Egypt was selected and identified according to Deshmukh[22] as *Thevetia peruviana*. Identification of the plant was further authenticated by Taxonomist Prof. Marei A. Hamed, in Faculty of Science, Al-Azhar University, Egypt. A voucher sample of *Thevetia peruviana* material has been deposited in the herbal collection of the plant and microbiology department, Faculty of Science, Al-Azhar University, Egypt.

Fifty grams of each Seed Kernel (SK) and Seed Pericarp (SP) were ground and extracted by methanol (200 mL) under continuous shaking for 10 hrs, then the methanolic extracts were filtered followed by evaporation. Extracts of plant samples were concentrated, followed by drying and then kept at -25°C till further investigation.

Analysis of SK and SP extracts by gas chromatography-mass spectrometry (GC-MS): The phytoconstituents analysis of SK and SP were performed by utilizing Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA). The existence of direct capillary column TG-5MS (30 m×0.25 mm×0.25 µm film thickness) characterizes this machine. The starting temperature of the column oven was held at 50°C, followed by temperature increment up to 230°C and then increased by 30°C per minute to maximum temperature up to 290°C which was isothermally constant for 2 min. Temperatures at 250 and 260°C were applied for keeping the injector and MS transfer, respectively; helium was applied carrier characterized by high purity at a rate of 1 mL min^{-1} at a constant flow. The cut time of solvent was set at 3 min, and one microliter of the diluted plant extract was injected via the Autosampler AS1300 joined with GC in the split mode. Using electron energy of 70 eV, the electron ionization mass spectra were collected in the range of m/z 40-1000 in full scan mode. Recognition of the extract constituents was done by assessing its mass spectra and Retention Time (RT), compared to the database of the National Institute of Standards and Technology (NIST) library mass spectral[23].

Determination of total content of phenolic and flavonoids: Total Phenolic Content (TPC) of SK and SP was assessed according to Folin-Ciocalteu method[24]. Extract of the plant (1 mL) was added to 5 mL of Folin-Ciocalteu reagent (diluted 10 fold) and 4 mL sodium carbonate solution (0.7 M). For one hour, the reaction mixture was incubated at 25°C temperature, following by measuring the absorbance at 765 nm using a spectrophotometer with a blank. Gallic acid at different levels ranged from 0.01-0.4 mg mL^{-1} dissolved in 1 mL methanol was applied as standard, following above mentioned procedure. TPC was expressed as gallic acid equivalents (mg of GAE g^{-1} sample) and calculated according to the following formula[24]:

$$TPC = \frac{GAC \times VP}{MP}$$

where, GAC is gallic acid concentration (mg mL^{-1}), VP is the volume of plant extract in mL and MP is methanolic plant extract weight in grams.

Total Flavonoids Content (TFC) of SK and SP was assessed according to the Aluminium chloride colorimetric method[24]. The reaction mixture contains 1 mL of plant extract and 3 mL of methanol followed by the addition of 0.2, 0.2 and 5.6 mL of aluminium chloride (10%), potassium acetate (1 M) and distilled water, respectively. Then the absorbance was recorded at 415 nm through using a spectrophotometer regarding a blank. Quercetin at different levels ranged from 25, 50, 100 and 200 µg mL^{-1} methanol were applied as standard, following above mentioned procedure. TFC was expressed as quercetin equivalent (mg of QE g^{-1} dw) and calculated according to the following formula:

$$TFC = \frac{QC \times VP}{MP}$$

where, QC is quercetin concentration (mg mL^{-1}), VP is the volume of plant extract in mL and MP is methanolic plant extract weight in grams.

Cell lines and cytotoxicity assay: The prostate cancer cells (PC-3) in the current study were obtained from Nawah Scientific Inc., Cairo, Egypt. PC-3 were preserved in DMEM media supplemented with streptomycin (100 mg mL^{-1}), penicillin (100 units mL^{-1}) and 10% of heat-inactivated fetal bovine serum, incubated in humidified containing 5% (v/v) CO_2 atmosphere at 37°C. Sulforhodamine B (SRB) assay was used for determining cell viability[25-26], briefly, aliquots of 100 µL cell suspension containing 5×10^3 cells were added to 96-well plates, followed by incubation for 24 hrs. Cells were treated with another aliquot of 100 µL media containing SK and SP extract at various concentrations. Cells exposure to treatments after 72 hrs were fixed by substituting media with 150 µL of 10% Trichloroacetic acid(TCA) and maintained for 1 hr at 4°C. The solution of TCA was detached and the cells were washed 5 times using distilled water. Aliquots of 70 µL SRB solution (0.4% w/v) were added and maintained in a dark for 10 min at room temperature. Using 1% of acetic acid, the plates were washed 3 times and allowed to air-dry for one night. Followed by the addition of 150 µL of TRIS base solution (10 mM, pH 10.5) to dissolve protein-bound SRB stain; the absorbance was recorded at 540 nm via a BMG LABTECH®- FLUOstar Omega microplate reader (Ortenberg, Germany).

Ferric reducing antioxidant power (FRAP) assay: Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) stock solution of 1 mM in methanol as standard for FRAP assay was prepared to utilize 10 serial dilutions that were prepared in the concentrations of 1000, 800, 600, 400, 200, 100 and 50 µM. These different concentrations of Trolox were applied for standard curve calibration. Dried extracts of SK and SP extracts were prepared in a concentration of 2 mg mL^{-1} in methanol. With slight alterations, the ferric reducing capacity assay was done to assess the total antioxidant activity of plant extracts as previously designated by Wojtunik-Kulesza[27]. A freshly reagent 2,4,6-tripyridyl-s-triazine (TPTZ) was prepared by mixing of 300 mM acetate buffer (pH = 3.6), a solution of 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3$ in a ratio of 10:1:1 (v/v/v, respectively). The reagent (190 µL) was mixed with sample solutions (10 µL), then added to each well (96 wells plate, n = 3), followed by keeping in the dark at room temperature for 30 min. The appeared blue colour as a result of the reduction of ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}) at the end of the incubation period was measured at 593 nm. The results were expressed as µmol Trolox equivalent/mg extract.

Antimicrobial activity of plant extract: Diffusion method using disc was used for assessing the activity of SK and SP extracts *in vitro* against some microorganisms. Antibacterial activity was planned against certain bacterial strains involving *Klebsiella pneumoniae* RCMB 003 (1), *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* RCMB 015 (1) and *Escherichia coli* ATCC 25922. The autoclaved nutrient agar was cooled at approximately 45°C, followed by bacterial inoculation under aseptic conditions, then poured into sterile petri dishes and allowed to solidify. Five mm diameter discs of filter paper (Whatman No. 1, India) were loaded with 20 µg per filter paper disc of crude extracts, then dried and placed on the prepared bacterial seeded agar layer and allowed to stand for1hr in the refrigerator for suitable diffusion of the extract. Then, the plates were incubated for 24 hrs at 37°C. The diameter of the inhibition zone (mm) around discs if appeared was measured to determine the antibacterial spectrum of extract. As mentioned above the antifungal activity of the extract was evaluated against *Cryptococcus neoformans* RCMB 0049001 and *Candida albicans* RCMB005003 (1) except using Yeast Extract Peptone Dextrose (YEPD) agar medium and the incubation period was at 35°C for 48 hrs[28]. Different antibiotics were provided from Sigma-Aldrich and used as positive control such as Amoxicillin/Clavulanic acid (30 µg), Cefoperazone (30 µg), Ampicillin (25 µg). While Ketoconazole (10 µg) was used as an antifungal agent. Discs were loaded by methanol as extracted solvent of plant samples was used also as control. All test organisms were taken from the Regional Centre for Mycology and Biotechnology (RCMB), Al-Azhar University, Egypt.

Ultrastructure examination by transmission electron microscopy (TEM): Ultrastructure of the sensitive tested organisms to potent extract (SK) was examined by TEM. The treated and untreated tested microbial cells were fixed using glutaraldehyde (3%), followed by washing in phosphate buffer, then fixed for 5min using a solution of potassium permanganate at 25°C. Prepared different dilutions of ethanol (10-90%) were for dehydrating the fixed samples for 15 min in each dilute separately gradually, followed by using absolute ethanol for 30 min. Via a graded series, the samples were infiltrated with epoxy resin and acetone till to finish in pure resin. The collected ultrathin sections on grids of copper were stained doubly in uranyl acetate, then using lead citrate. TEM (JEOL-JEM 1010, USA) at 70 kV was used to examine the ultrastructure stained sections at RCMB[29]. All methods were performed per the relevant guidelines and regulations.

RESULTS

During plant collection, it shows that the green fruits of *T. peruviana* (Fig. 1a) become black at the ripening stage. The nut of each fruit is characterized by longitudinal and transverse divide (Fig. 1b). Phytochemical analysis by GC-MS showed variation and differences in chemical profiles of SK and SP (Tables 1-2 and Fig. 2-3). The detected compounds in SK was more than detected in SP. Certain ingredients were detected in SK but not in SP such as Isophorone, (Z)-Tagetenone, Camphor, endo-Borneol, α-Terpineol, (-)-Carvone and Bicyclo[2.2.1.]Heptan-2-OL,1,7,7-trimethyl-, Acetate. Borneol and Bicyclo[2.2.1.]Heptan-2-OL,1,7,7-Trimethyl-, Acetate or its derivatives. Other biological active compounds such as a sesquiterpene (Spathulenol), was reported as a constituent of SK extract of *T. peruviana* (Table 1). The methanolic extract of SP contains 13 constituents (Table 2). In the present study,3',4',7-Trimethylquercetin,10-Octadecenal and Methyl 9-cis,11-trans-octadecadienoate were detected in SP extract (Table 2). A comparison between the total contents of phenolic and flavonoid of SK and SP of *T. peruviana* extract

Fig. 1: *T. peruviana* (a) arrow directed to fruits and (b) seeds

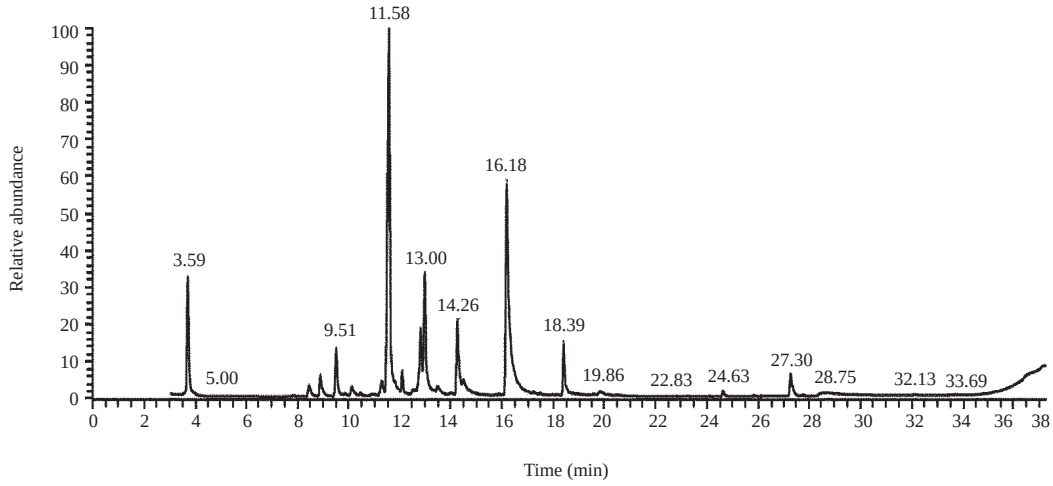

Fig. 2: Chromatogram analysis of *T. peruviana* seed kernel extract by GC-MS

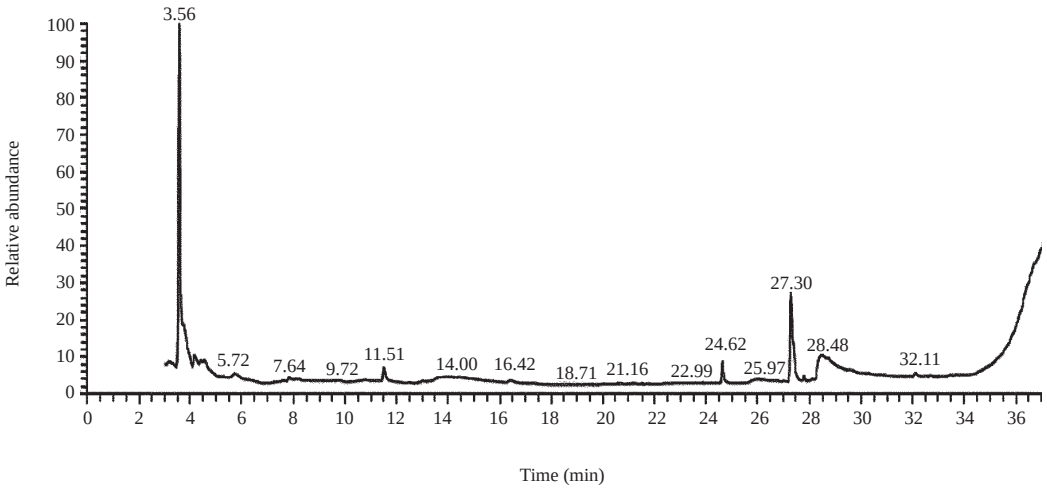

Fig. 3: Chromatogram analysis of *T. peruviana* seed pericarp extract by GC-MS

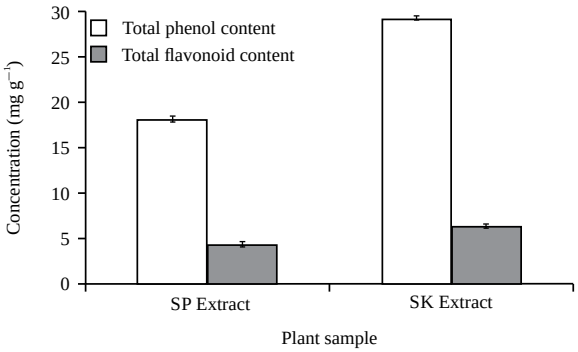

Fig. 4: Total phenol mg GAE g^{-1} and flavonoid contents mg QE g^{-1} dry weight of seed kernel and seed pericarp extracts

Table 1: Chemical constituents of seed kernel extract of *T. peruviana* by GC-MS

Compound name	R.T.	Area (%)	M.F.	M.W
1-Methoxy-2-propyl acetate	3.69	9.10	$C_6H_{12}O_3$	132
Isophorone	8.44	1.10	$C_9H_{14}O$	138
Camphor	8.89	1.76	$C_{10}H_{16}O$	152
endo-Borneol	9.51	3.81	$C_{10}H_{18}O$	154
a-Terpineol	10.11	1.02	$C_{10}H_{18}O$	154
(-)-Carvone	11.28	1.42	$C_{10}H_{14}O$	150
2-Isopropyl-5-Methyl-3-Cyclohexen-1-one	11.57	35.52	$C_{10}H_{16}O$	152
Bicyclo[2.2.1]Heptan-2-OL,1,7,7-Trimethyl-, Acetate,Endo-	12.13	1.53	$C_{12}H_{20}O_2$	196
(Z)-Tagetenone	12.84	4.24	$C_{10}H_{14}O$	150
Chrysanthenone	13.00	8.15	C_9H_{14}	122
2-Pinen-7-one	13.00	8.15	$C_{10}H_{14}O$	150
p-Mentha-1,4(8)-dien-3-one	13.50	0.70	$C_{10}H_{14}O$	150
2-Propenoic Acid, 3-Phenyl-, Methyl Ester	14.49	0.60	$C_{10}H_{10}O_2$	162
2-Propenoic Acid, 3-Phenyl-, Ethyl Ester	16.18	17.91	$C_{11}H_{12}O_2$	176
(-)-Spathulenol	18.39	4.29	$C_{15}H_{24}O$	220
Methyl Hexadecanoate	24.63	0.49	$C_{17}H_{34}O$	270
Methyl 9-cis,11-trans-octadecadienoate	27.30	2.19	$C_{19}H_{34}O_2$	294

R.T: Retention time, M.F: Molecular formula, M.W: Molecular weight

Table 2: Chemical constituents of seed pericarp extract of *T. peruviana* by GC-MS

Compound name	R.T.	Area (%)	M.F.	M.W
1-Methoxy-2-propyl acetate	3.56	54.02	$C_6H_{12}O_3$	132
Pyrimido[1,2-a]azepine, 2,3,4,6,7,8,9,10-octahydro-	3.76	1.13	$C_9H_{16}N_2$	152
2-Furanmethanol	4.15	3.61	$C_5H_6O_2$	98
Levoglucosenone	4.42	1.19	$C_6H_6O_3$	154
10-Octadecenal	4.55	2.25	$C_{18}H_{34}O$	266
p-Menth-1-en-3-one	11.51	2.47	$C_{10}H_{16}O$	152
Methyl Hexadecanoate	24.62	4.17	$C_{17}H_{34}O_2$	270
Methyl 9-cis,11-trans-octadecadienoate	27.30	18.93	$C_{19}H_{34}O_2$	294
Methyl-9,9,10,10-D4-Octadecanoate	27.79	0.87	$C_{19}H_{34}D_4O_2$	302
cis-9,cis-12-Octadecadienoic acid	28.39	7.72	$C_{21}H_{38}O_2$	322
6,8-DI-C-á-Glucosylluteolin	36.44	1.28	$C_{27}H_{30}O_{16}$	610
3',4',7-Trimethylquercetin	36.71	1.33	$C_{18}H_{16}O_7$	344
Ethyl Iso-allocholate	37.37	1.02	$C_{26}H_{44}O_5$	436

R.T: Retention time, M.F: Molecular formula, M.W: Molecular weight

was recorded (Fig. 4). SK extract was rich with phenolic and flavonoid contents 29.25 and 6.34 mg g^{-1} dry weight compared with the content of SP.

From the obtained results, the cytotoxicity of *T. peruviana* methanolic extract against prostate cancer increment with Increasing the concentration of extract (Table 3 and Fig. 5). A remarkable reduction of the PC-3 cell line was observed with SK extract compared with SP extract with IC_{50} values 0.05 and 35 µg mL^{-1}, respectively. Resistance (%) of PC-3 viability to SK was 0% while it was 34% to SP extract (Table 3). In the current study, the morphological changes of PC-3 treated by *T. peruviana* SK extract were observed (Fig. 6a-f). Low concentrations of SK extract induced low deformation in cells (Fig. 6b), then this deformation

Table 3: Viability and Cytotoxicity of seed kernel and seed pericarp extracts against PC-3

	SK(%)		SP	
Concentration (μg mL^{-1})	Viability (%)	Toxicity (%)	Viability (%)	Toxicity (%)
0.0	100±0.0	0.0	100±0.0	0.0
0.1	55.60±2.47	44.40	97.56±2.47	2.44
1.0	35.62±1.87	64.38	97.13±1.99	2.87
10	29.60±1.04	70.40	93.95±1.53	6.05
100	29.09±1.93	70.91	43.47±0.80	56.53
1000	28.80±0.21	71.20	34.89±2.45	65.11
IC_{50} μg mL^{-1}	0.05		35.0	
Resistance (%)	0.0		34.0	

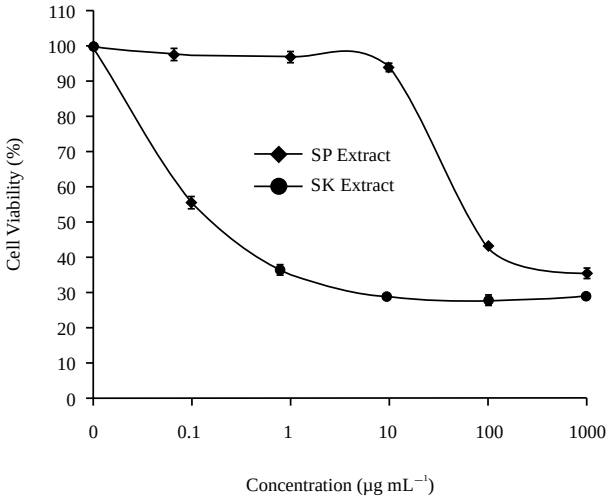

Fig. 5: Cytotoxicity of seed kernel and seed pericarp extracts against PC-3

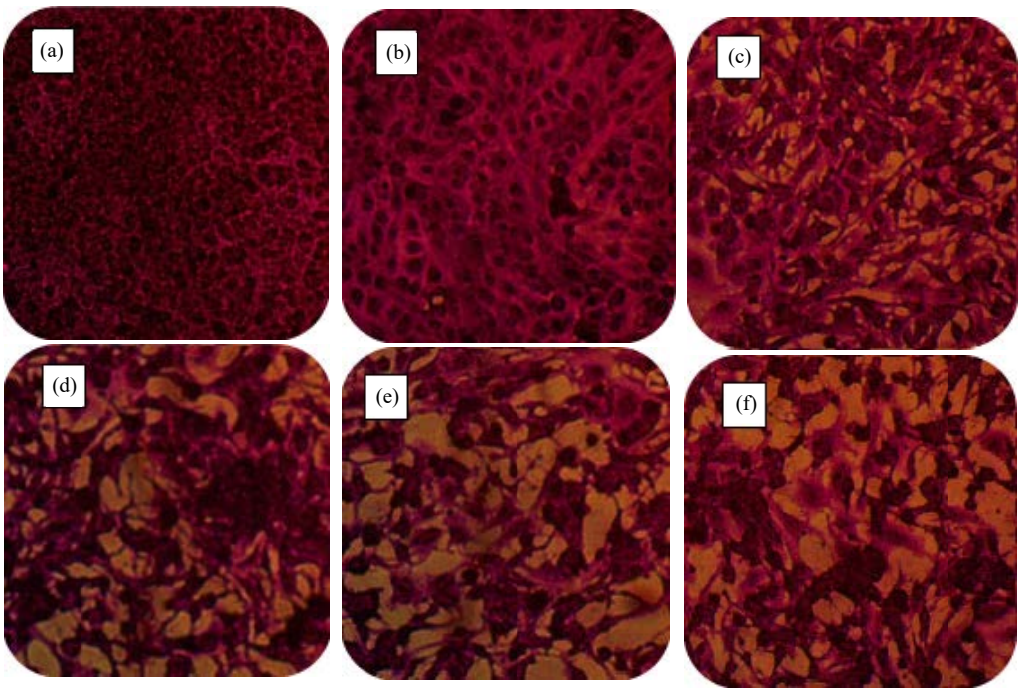

Fig. 6: Morphological alteration of PC-3 treated by different concentrations of seed kernel extract, a: Control, b: 0.1 μg, c: 1 μg, d: 10 μg, e: 100 μg and f: 1000 μg

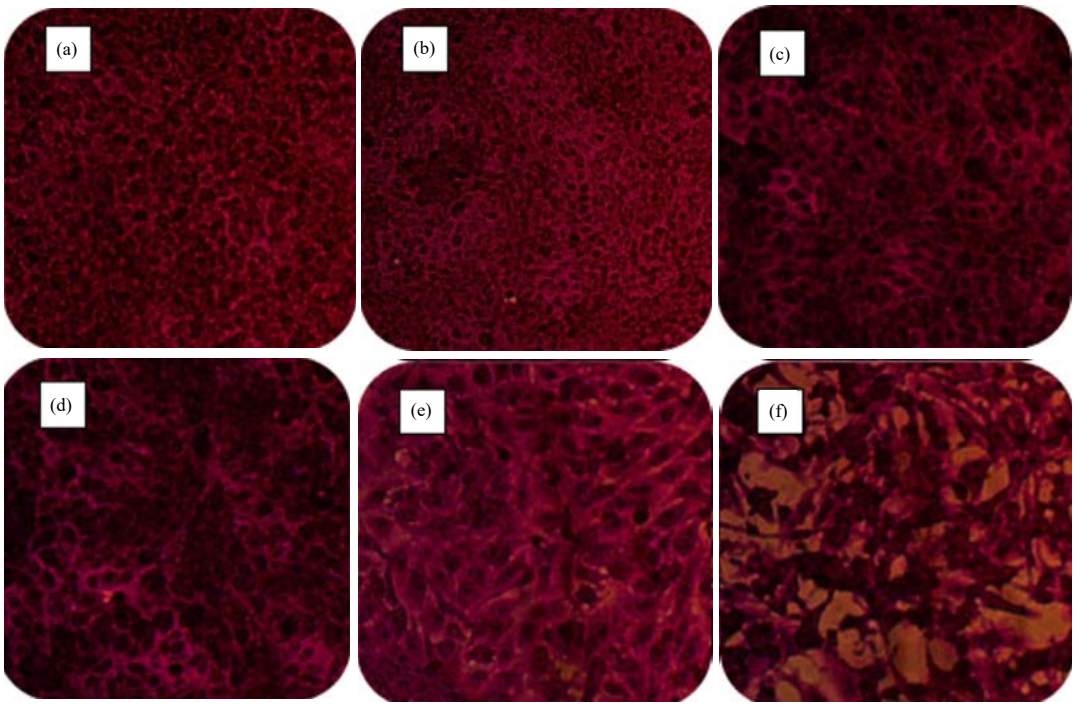

Fig. 7: Morphological alteration of PC-3 treated by different concentrations of seed pericarp extracts, a: Control, b: 0.1 µg, c: 1 µg, d: 10 µg; e: 100 µg and f: 1000 µg

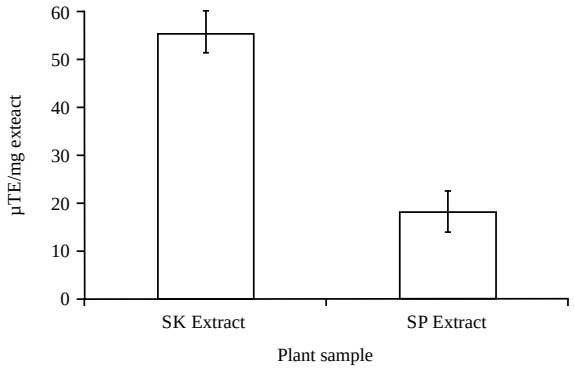

Fig. 8: Antioxidant of seed kernel and seed pericarp extracts (µmTE mg^{-1} extract)

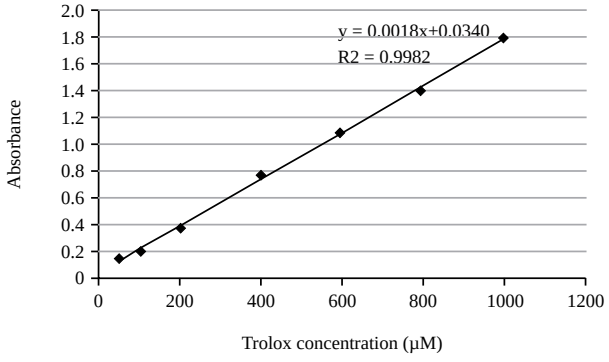

Fig. 9: Linearity of Trolox in FRAP assay

increment with treatment concentration increased (Fig. 6c-d) and become clearly at high concentrations (Fig. 6e-f) unlike control treatment (Fig. 6a). On the other hand, compared with control (Fig. 7a), no appeared deformation was observed (Fig. 7b-d) at low concentrations of SP extract (0.1, 1, 10 and 100 µg mL^{-1}) but weak deformation was observed at 100 µg mL^{-1} (Fig. 7e). Morphological characteristics of PC-3 treated with 1000 µg mL^{-1} of SP extract was more affected (Fig. 7f). Depending on treatment concentration, diverse degrees of PC-3 cells changes comprising membrane shrinkage, blebbing of the cell membrane, failure of cell adhesion, appearance of unusual cellular crinkle and cell destruction were microscopically recorded particularly with using SK extract.

SP and SK of *T. peruviana* extracts exhibited antioxidant activity, but the highest antioxidant activity (55.55 µmTE mg^{-1} extract) was associated with SK extract compared with SP extract (18.19 µmTE mg^{-1} extract) with to nearly three folding increase (Fig. 8). These results were measured regarding the calibration curve of Trolox in FRAP assay (Fig. 9). The values are directly interrelated with antioxidant activity as the higher the FRAP value the greater is the antioxidant activity.

The current result reflected the antimicrobial activity of SK extract against tested bacteria and yeasts, but with varying

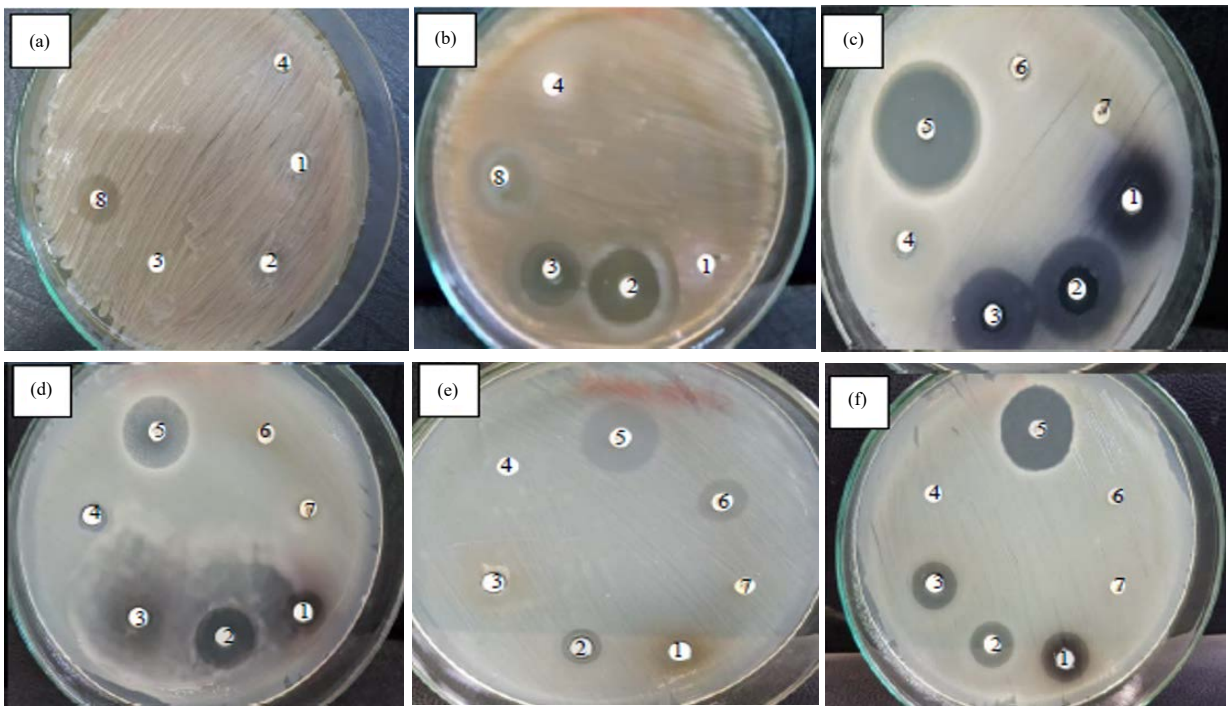

Fig. 10: Antimicrobial activity of Seed Kernel (SK) and Seed Pericarp (SP) against bacteria and yeasts, a: *C. neoformans*, b: *C. albicans*, c: *B. subtilis*, d: *K. pneumoniae*, e: *S. aureus* and f: *E. col*

1: SP, 2: SK, 3: SP+SK, 4: Methanol solvent, 5: Amoxicillin/Clavulanic acid (30 µg), 6: Cefoperazone (30 µg), 7: Ampicillin (25 µg) and 8: Ketoconazole (10 µg)

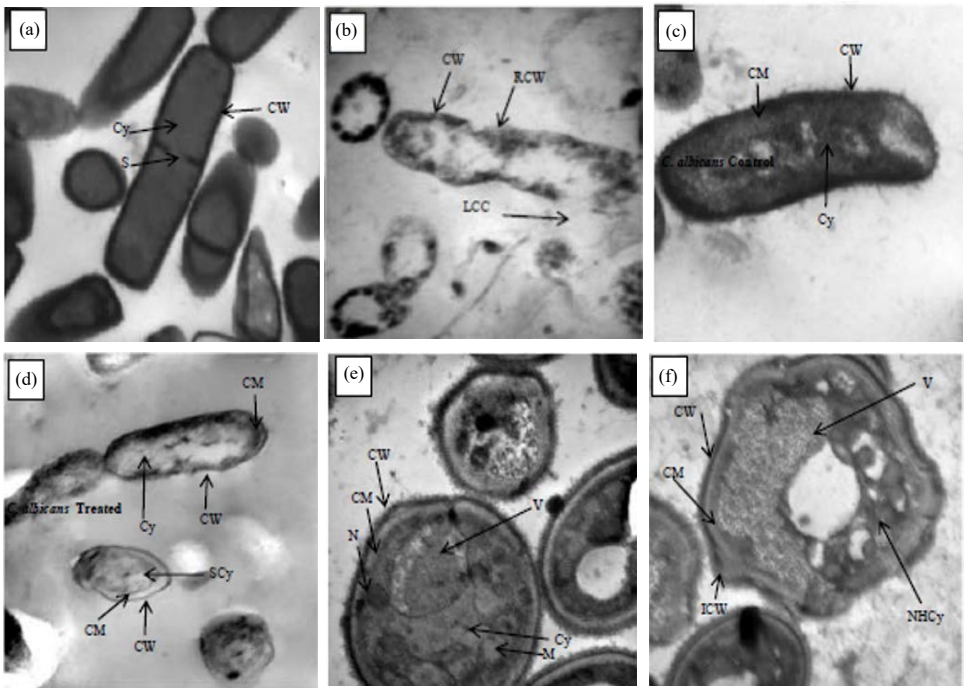

Fig. 11a-f: Transmission electron microscopic morphology of *B. subtilis*, *K. pneumoniae* and *C. albicans* treated by seed kernel extract

CW: Cell wall, ICW: Irregular cell wall, RCW: Rupture cell wall, S: Septum, SCy: Shrinkage cytoplasm, N: Nucleus, V: Vacuole, M: Mitochondria, CM: Cell membrane, NHCy: Nonhomogeneous cytoplasm and LCC: Leakage of cell constituents

Table 4: Antimicrobial activity of Seed Kernel (SK) and Seed Pericarp (SP) extracts

	Inhibition zone (mm)					
	Bacteria				Yeasts	
Treatment	*B. subtilis*	*K. pneumoniae*	*S. aureus*	*E. coli*	*C. albicans*	*C. neoformans*
SP	0.0±0.0	14±0.40	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
SK	15.2±0.4	20.4±1.5	12. 3±0.4	15.6±0.40	30.4±0.2	0.0±0.0
SK+SP	10.5±0.3	14.3±0.5	0.0±0.0	15.5±0.3	25.2±0.5	0.0±0.0
Methanol	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
AMC	35.3±1.2	24.5±0.2	20.2±0.4	28.3±0.2	-	-
CEP	0.0±0.0	0.0±0.0	14.6±0.3	0.0±0.0	-	-
AMP	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	-	-
KTC	-	-	-	-	12.0±0.25	15±0.33

AMC: Amoxicillin/Clavulanic acid (30 µg), CEP: Cefoperazone (30 µg), AMP: Ampicillin (25 µg) and KTC: Ketoconazole (10 µg)

degrees of inhibition depending on species (Table 4 and Fig. 10a-f). *C. neoformans* (Fig. 10a) growth not inhibited by extracts while *C. albicans* (Fig. 10b) was highly sensitive to SK extract of *T. peruviana* extract, while no synergistic effect appeared as a result of a combination between the extract of SK and SP (Table 4). *K. pneumoniae* (Fig. 10e) was more resistant than *B. subtilis* (Fig. 10c) to the antibiotic used but the SK extract was more efficient on *K. pneumoniae* (20 mm inhibition zone) compared with the other tested bacteria *S. aureus* and *E. col* (Fig. 10e-f).

The effect of SK extract on *K. pneumoniae*, *B. subtilis* and *C. albicans* ultrastructures was investigated (Fig. 11a-f). There were major ultrastructural alterations of treated cells compared to untreated cells (Fig. 11a-f). Treated cells of *B. subtilis* had chromatin compression which was crowded into apoptotic-like particles, besides the observed alteration including breakage of the cell wall. Another change was observed in *K. pneumoniae* where the cell membrane was detached from the cell wall. Treated *C. albicans* exhibited limited changes in its ultrastructures included irregular cell membrane (Fig. 11a-f).

DISCUSSION

In the current study, the detected compounds in SK was more than detected in SP. Chemical ingredients of *T. peruviana* leaves extract was reported in many studies but few studies have focused on the chemical ingredients of fruits. Several active molecules with different biological activities such as steroids, acids, esters, alcohols, alkaloids and nitro compounds were detected in plant extract[30]. Most of the detected compounds in SK and SP extracts according to previous studies increased nasal and gastrointestinal bioavailability of drugs, circulation of drugs in tissues of the brain[31]. Moreover, its antimicrobial, anti-inflammatory and antiviral activity were reported[32,33]. The related compounds to terpenes such as Camphor and 2-Pinen-7-one were detected in the essential oil of *T. peruviana* flowers[34]. Another recent study indicated that GC-MS investigation of essential oil extracted from flowers, leaves and fruits of *T. peruviana* reflected the presence of monoterpenes, sesquiterpenes, terpenoids and sterol[35]. A sesquiterpene (Spathulenol), was reported as a main volatile ingredient of numerous aromatic Myrtaceae plants[36]. Several biological activities such as antiproliferative[37] and antimicrobial activities[38] and anti-inflammatory[39] were documented to spathulenol that detected in of SK extract of *T. peruviana*. The problem of *T. peruviana* is concomitant to the presence of toxic compounds as confirmed previously. Kohls *et al.*[40] recorded the presence of six cardiac glycosides in the *T. peruviana* extract of seeds. GC/MS analysis recognized 3',4',7-Trimethylquercetin in SP extract (Table 2), Quercetin and its derivatives were identified previously in methanolic extract of *T. peruviana* leaves[11] and recorded responsibility for antifertility potential. Some of the identified constituents in the current finding such as 10-Octadecenal have been informed to possess vital activities previously[41]. Methyl 9-cis,11-trans-octadecadienoate was identified in SK and SP, some author it's known as conjugated linoleic acid and functionalized as anticarcinogenic and anticholesterolemic agent[42]. GC-MS analysis results suggest that extract of SK are efficient than SP extract from *T. peruviana* with various bioactivity. As mentioned in previous studies, flavonoids and phenolic of plant extracts perform a critical role in biological activities such as antioxidant, anticancer and antimicrobial. From the obtained results, SK extract was richer with phenolic and flavonoid contents than SP (Fig. 4). The total content of phenolic and flavonoid in *T. peruviana* was varied with the plant organ, these finding was documented previously[18], where fruits pose highest content of phenolic and flavonoid, followed by leaves and roots extract.

Anticancer of *T. peruviana* methanolic extract against prostate cancer was documented in the current finding (Table 3 and Fig. 5) with low IC_{50} particularly SK extract (0.05 µg mL^{-1}). The previous study recorded the anticancer activity of the *T. peruviana* methanolic extract against numerous cancer cell lines of human[15], including breast, PC-3, lung and colorectal cells with different IC_{50} values based on cell type but not exceed for 12.04 µg mL^{-1}. Recently El-Sawi *et al.*[16] documented the anticancer activity of *T. peruviana* using breast MCF7 and liver HEPG2 carcinoma cell lines. Extract of essential oil for various parts of *T. peruviana* including flowers, fruits and leaves was examined and exhibited inhibitor efficacy against five cancer cell lines[35], which may due to the presence of monoterpenes, sesquiterpenes, terpenoids and sterol. Depending on treatment concentration, diverse degrees of PC-3 cells changes (Fig. 6a-f and 7a-f) comprising membrane shrinkage, blebbing of the cell membrane, failure of cell adhesion, appearance of unusual cellular crinkle and cell destruction were microscopically recorded particularly with using SK extract. The current finding was in agreement with published results[15], where membrane blebbing and detachment of cells were recorded. El-Sawi *et al.*[16] mentioned that *T. peruviana* has no mutagenic influence against somatic or germ cells. Induction of apoptotic cell death was observed by treatment by *T. peruviana* extract, these mechanisms were established by DNA fragmentation[15].

SK extract of *T. peruviana* reflected the highest antioxidant activity (55.55 µmTE mg^{-1} extract), however, SP possesses also antioxidant activity. El-Sawi *et al.*[16] mentioned that *T. peruviana* displayed antioxidant activity with 120.098 mg g^{-1} extract, designed as Trolox equivalent (TE g^{-1}) for FRAP assay. The differences in the level of the antioxidant activity of *T. peruviana* may depend on the plant organ used either leaves or stems or fruits, geographical region of plant cultivation. All these parameters may affect the plant contents of active ingredients. In a recent study, the antioxidant activity of *T. peruviana* leaves extracts was reported[21], due to the existence of different phytochemicals in leaves. It is established that *T. peruviana* leaves and fruits extracts possess antioxidant and anti-inflammatory action based wound healing proper. Fruit extract of *T. peruviana* showed the highest wound breaking strength than leaves extract. Based on therapeutic approaches including antioxidant, antimicrobial and anti-inflammatory activities[43].

From the antimicrobial test, SK extract was more effective against tested bacteria and yeasts compared with SP extract depending also on species (Table 4 and Fig. 10). For example, *K. pneumoniae* was more inhibited (20 mm inhibition zone) by SK extract unlike other tested bacteria, however *K. pneumoniae* was highly resistant to used antibiotic (Amoxicillin/Clavulanic Acid30 µg). The obtained results were in partial agreement with Hassan *et al.*[6] explanation of bactericidal activity of *T. peruviana* ethanolic leaves extract test against *Staphylococcus aureus*, *Salmonella typhi*, *Shigella flexineri*, *Klebsiella* sp, beside *Shigella sonnei*. Effective antibacterial potential of aqueous and ethanol extracts of *T. peruviana* fruits and flowers was observed[18]. Also, Nesy and Lizzy[14] recorded the inhibitory activity of *T. peruviana* seed kernels against *Pseudomonas aeruginosa* (18.67 mm) and *Nocardia asteroids* (15.00 mm). Surprisingly *C. albicans* was inhibited but *C. neoformans* growth was not inhibited by SK and SP extracts. The fungicidal potential of the *T. peruviana* seeds extract was reported previously against unicellular and filamentous fungi[14,44]. Recently Meena *et al.*[45] studied the phytoconstituents and biocidal potential of *T. peruviana* leaves extract against *Alternaria solani* and recorded the greatest inhibitory activity. Different degree of antibacterial activity of *T. peruviana* leaves extract depending on the used solvent for extraction and bacterial species was documented[20], where low activity was recorded against *Micrococcus luteus* and *Shigella flexneri* while high activity was recorded against *P. aeruginosa* using methanolic extract of leaves, the moderate activity of acetone extract was recorded against *P. aeruginosa*, *M. luteus* and *S. flexneri*. In contrast, moderate action of hexane extract was observed against *M. luteus*, low activity against *P. aeruginosa* and *Shigella flexneri* was recorded. The ultrastructural study reported numerous changes in bacterial and yeast cell structure (Fig. 11) that were exposed to SK extract of *T. peruviana*, these changes may help to identify the antibacterial action mechanism of SK extract. The observed alteration may be due to the efficacy of SK extract on bacterial metabolism that directly or indirectly managed the ultrastructure of cells. Cell membrane collapsed out the cell wall and irregular appearance of *E. coli* resulted from the effect of *Syzygium aromaticum*[4]. *Syzygium legatii* and *Eugenia zeyheri*[5]. Partial changes in ultrastructures of *C. albicans* exposed to SK extract was observed in the current results, however, Rella *et al.*[46] explained the changes of the fungal cell membrane exposed to plant extract associated with the microdomains composition of the lipid. Finally, the biological activities of SK extract provide a scope for the further investigation of these extracts for various therapeutic properties and their use as a potent drug for cancer treatment and inhibit the growth of multi-drug resistance microbes. However, care should be taken as the plant has poisonous properties as mentioned in earlier reports.

CONCLUSION

GC-MS profile of the plant indicated that numerous chemical constituents are existed in SK compared with SP extract. SK extract showed high cytotoxicity against PC-3 cell line with low IC_{50} (0.05 μg mL^{-1}), while the IC_{50} of SP was 35 μg mL^{-1}. The antioxidant activity of SK was higher than SP with three folding times. The antimicrobial activity was observed using SK extract while SP extract showed no or negligible inhibition on certain bacteria.

SIGNIFICANCE STATEMENT

This study discovers the phytoconstituents of *Thevetia peruviana* extract by GC-MS that can be applied for cancer and human microbial pathogens (*S. aureus*, *K. pneumoniae*, *B. subtilis*, *E. coli*, *C. albicans* and *C. neoformans*) control. This study will help the researcher to uncover the critical area of cancer treatment and prevent microbial infections by natural products that many researchers were not able to explore. Thus, a new theory on these natural constituents applications may be arrived at.

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