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Comparative studies of anticancer, antimicrobial, antidiabetic, antioxidant activities of *Daphne oleoides* and *Berberis baluchistanica* extracts native to Pakistan

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Abstract: To investigate *in vitro* anticancer, antimicrobial, antioxidant and in vivo hypoglycaemic effects of crude methanolic extracts (CMEs) of *Berberis baluchistanica* and *Daphne oleoides*. MTT assay for cytotoxicity on HeLa and NIH cells, disc diffusion protocols for antimicrobial and DPPH assay for antioxidant potential were applied. *In vivo* hypoglycaemic effect was investigated on Alloxan-induced diabetic rabbits. *D. oleoides* CME exhibited moderate cytotoxic behaviour against HeLa cells (IC_{50} 77.87μg/mL) whereas *B. baluchistanica* CME was found deficient (IC_{50} 170.02μg/mL). *P. aeruginosa* was susceptible to both CMEs. *M. luteus* and *B. subtilis* was prone to the bactericidal effects of *D. oleoides and B. baluchistanica* CMEs respectively. *D. oleoides* CME inhibited more than 80% *S. cerevisiae* and 60% *C. glabrata* colonies. *B. baluchistanica* CME showed significant antioxidant activity (IC_{50} 52.86μg/ml) than *D. oleoides* CME (IC_{50} 87.30μg/ml) and standard resveratrol (IC_{50} 109.46μg/ml). *B. baluchistanica* CME showed superior antidiabetic effect (135.75 mg/dl ±0.53) as compared to *D. oleoides* CME (191.50 mg/dl ± 0.48) but less antidiabetic effect than metformin hydrochloride (standard). All the above potentials exhibited by *D. oleoides and B. baluchistanica* CMEs propose further investigations to isolate and purify responsible biologically active lead molecule(s) with diverse capabilities.

Keywords: Alloxan–induced diabetic rabbits, anticancer, antimicrobial, antioxidant, *Berberis baluchistanica*, *Daphne oleoides*, *in-vivo* antidiabetic activity, MTT assay.

INTRODUCTION

Plants are the richest source of potent drugs with enormous diversity in biological potential. Baluchistan (Pakistan) due to its upper and lower high lands, plains and deserts have wide range of medicinal plants used extensively in traditional and ethnic groups. Thymelaeaceae is cosmopolitan family of flowering plants. 5 genera and 7 species are distributed in different areas of Pakistan (Zaidi *et al.,* 2015). Berberidaceae is widely distributed family of medicinal plants. In Pakistan 31 species of Berberidaceae were divided in to 3 genera. Plants of genus Berberis (Berberidaceae) contain 29 species that have been identified in Pakistan (Jafri *et al.,* 1973). *D. oleoides* extract displayed wound healing potential and further isolation disclosed quercetin3-*O*-glucoside as potential wound healing agent (Süntar *et al.,* 2014), it also possesses potent anti-nociceptive activities (Kupeli *et al.,* 2007) inhibiting biosynthesis of inflammatory response factors like cytokines, interleukin and tumour necrosis factor–*α* (Yesilada *et al.,* 1997). It also owned gut modulatory activities by cholinergic and calcium channel blocking mechanism (Khan *et al.,* 2011). *D. oleoides* CME contains di and tri-meric coumarins that are potent inhibitor of urease enzyme. Urease accelerates the urolithiasis, hepatic encephalopathy, nephrotoxicity and provides favourable pH environment for *Helicobacter pylori* to cause peptic and gastric ulcers (Mobely and Hausinger, 1989). Different chemical assay was performed to demonstrate strong antioxidant potential of crude extracts of *D. oleoides* (Zengin *et al.,* 2013). Daphnae species contain potent anticancer diterpenes effective against various cancer cell lines (Jiang *et al.,* 2015).

Thymelaeaceae species contain the structurally closely related polycyclic skeletons of diterpene esters known as tetracyclic (Tigliane and Ingenane) and the tricyclic daphnane. *Daphne* species contain daphnane-type diterpene that is well known for its ethno pharmacological importance and has been employed in conventional medication for the treatment of various diseases (Niwa *et al.,* 1982). A structural modification of daphnane-type diterpene produces potent phorbol ester e.g. prostratin (Miana *et al.,* 1985) exhibiting irritant, toxic, carcinogenic as well as antileukemic activities. Compounds of various classes like coumarins, flavonoids, lignin, steroids, glycosides and polyphenols were also extracted from *D. oleoides* (Riaz *et al.,* 2016).

Different biological activities have also been reported for Berberis species that include antimicrobial, antihyperglycemic, antioxidant, anti-inflammatory, anticancer and wound healing. Traditionally, berberis

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berries extract was used as blood purifier (Karimov, 1993).Berberine and berbamine isolated from Berberis plants have antioxidant, anti-hyperglycaemic, anti-inflammatory, heptoprotective and hypotensive properties (Končić *et al.,* 2010). Different parts of *Berberis vulgaris* showed anti-Inflammatory, antimicrobial, urolithiasis, anti-diabetic, antihistaminic, anticholinergic and fertility related activities. Berberine and berbamine isolated from *Berberis lycium* and *Berberis orthobotroys* exhibited cardiovascular potentials (Srivastava *et al.,* 2015). *Berberis lycium* CME of roots demonstrated a good wound healing activity (Khan *et al.,* 2016). Antileishmanial, cytotoxic and antioxidant activities of *B. baluchistanica* were also reported (Baloch *et al.,* 2013). Various phytochemicals of diverse classes (alkaloids, flavanoids, terpenoids, anthocyanins, sterols, vitamins, lignins, carotenoids, proteins and lipids) were isolated from different parts of berberis plants. Alkaloids (berberine, berbamine, baluchistanamine, thalifoline and isotetrandrine), polyphenolic flavonoids (caffeic acid, quercetin, meratin, chlorogenic acid and rutin), various other nutrients (β-carotene, anthocyanin and ascorbic acid) and minerals were obtained from different parts of berberis plants (Srivastava *et al.,* 2015). Current study is a comparative evaluation of different pharmacological properties of *D. oleoides and B. baluchistanica* indigenous to Baluchistan, province of Pakistan. It will be helpful to open a new horizon in developing a therapeutically active lead(s) molecule with pronounced activity.

MATERIALS AND METHODS

Plant collection and extraction

B. baluchistanica (Ref. No. DOF-MBD-0024) stems and *D. oleoides* (Ref. No. DOF-MBD-0025) roots were identified and collected from Quetta Baluchistan (Pakistan) by kind cooperation of Mr Irshadullah Gondal District Officer Forest Mandi-Bahauddin Pakistan. The plant was washed and dried in shade. Turned into small pieces grinded to fine powder and stored in air-tight container at room temperature.

Grinded powder of *B. baluchistanica* (2.0 Kg) and *D. oleoides* (2.0 Kg) was placed in separate percolators and soaked in 10.0 litres of methanol. After 2 weeks methanolic extracts were collected and subjected to rotary vacuum evaporator to obtain concentrated extracts. The percent yield for *B. baluchistanica* CME and *D. oleoides* CME was 65% and 56% respectively.

Biological activities

Cytotoxic activity

MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) assay was performed to investigate the *in vitro* toxicity profile of *D. oleoides* and *B. baluchistanica* CMEs using HeLa (Cervical Cancer cell line) and NIH/3T3 (*Mus musculus* embryonic fibroblast cell) obtained from ICCBS, university of Karachi-Pakistan . MTT colorimetric assay (IC_{50} value) is used to determine the cytotoxic effects, cell proliferation and viability of cells (Mosmann, 1983).

MTT assay-principle

Microculture Tetrazolium (MTT) is a colorimetric assay for evaluating cell metabolic activity. Mitochondria succinate dehydrogenase enzymes, under defined conditions, reflect the number of viable cells present. The enzymes reduces the tetrazolium dye MTT to its insoluble purple color formazan (chromophore complex), measured spectrophotometrically (Mosmann, 1983). Conversion of MTT to formazan occurs only in living cell so viable cell count can be related to formation of formazan.

Procedure

HeLa and NIH cell lines were cultured in 10% PBS (phosphate buffer saline) containing DME (Dulbecco's modified eagle) medium accompanied by penicillin (50 units / ml) and streptomycin (50 units / ml). Incubation conditions were set to be 37 °C in humidified CO_2 (10%). HeLa and NIH cells were inoculated with the density 6 x 10^4 and 6 x 10^3 cells in 96 well microtiter plates respectively containing 200µl culture medium. Samples of *D. oleoides* and *B. baluchistanica* CMEs were separately dissolved in dimethylsulfoxide (DMSO), dilution of 25, 50, 100 and 200µg/ml in medium from stock solutions (10mM) of *D. oleoides*, *B. baluchistanica*, doxorubicin and cyclohexamide were prepared. Doxorubicin and cyclohexamide were used as positive control for HeLa and NIH/3T3 cell lines respectively. After 24 hours 10µl each concentration of plant extracts, positive control (containing standard drugs) and negative control was transferred to wells containing cells. Microtiter plates were incubated at 37°C, in humidified CO_2 (10%) incubator for 72 hours. After incubation, medium was removed from the wells and 150 µl of fresh DME medium and 50 µl of MTT solution (0.2% in phosphate buffered saline pH 7.4) was added in each well and further incubated for 4 hours in same conditions. Medium and MTT solution was removed and insoluble formazan purple crystals were dissolved in 50 µl in DMSO. Absorbance was measured at 570nm in microtiter plate's reader (EL x 808, BioTek, USA). Mean and standard deviation of hexaruplicate were determined. IC_{50} values were calculated by sigma plot and toxicity (percentage of cells inhibited) and cell viability (percentage of cells survived) was measured by the following formula:

Percentage cell inhibition =100 -Percentage cell Survival

Percentage cell Survival = $(A_T - A_B) / (A_C - A_B) \times 100$

Where

A_T = Absorbance of test sample

A_B = Absorbance of blank

A_C = Absorbance of control sample

Antimicrobial activity

Microbial strains and reference drugs

Different bacterial strains *Salmonella typhi* (ATCC 14028), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027), *Stenotropomonas maltophilia* (ATCC 13637), *Bacillus subtilis* (ATCC 5230), *Micrococcus luteus* (ATCC 9341 and fungal strains *Saccharomyces cerevisiae* (ATCC 9763), *Candida glabrata* (ATCC 90030) and *Candida albicans* (ATCC 10261) were obtained from Amson Vaccines & Pharma Islamabad and Riphah international University Islamabad. Gemifloxacin mesylate and nystatin standard drugs were provided by Amson Vaccines & Pharma Islamabad.

Preparation of reference and sample solution

Stock solutions (300µg/ml) of *B. baluchistanica* CME and *D. oleoides* CME, gemifloxacin mesylate and nystatin reference drugs were prepared in methanol separately. Each stock solution was further diluted to 30, 60 & 90 µg/ml concentrations in methanol respectively.

Preparation of microbial culture

Bacterial strains were cultured in nutrient broth and incubated at 37°C for 48 hours and fungal strains were cultured in Sabouraud dextrose agar, incubated at 28°C for seven days. Inoculums of each bacterial and fungal strain were prepared in 10 ml normal saline solution. The bacterial OD_{600} (Optical density) and fungal OD_{530} suspension were compared to the 0.5 McFarland standards.

Preparation of media and cultured plates

28g of nutrient agar and 65 grams of Sabouraud dextrose agar was suspended in 1 litre of distilled water separately. The suspensions were heated to dissolve the medium completely and then autoclaved at 121°C for 15 minutes. Cooled to about 45°C before transferring to petri plates. Media was allowed to solidify in petri plates on sterile surface. The petri dishes were incubated at 37 °C for 24 hours for nutrient agar and 7 days for sabouraud dextrose agar to check any growth. Each bacterial and fungal strain culture was inoculated on three different petri plates separately.

Disc diffusion method

Disc diffusion method (Kirby-Bauer method) was used to test the antimicrobial potential of *D. oleoides* and *B. baluchistanica* CMEs (Bauer, 1966). Commercially available sterile discs (4.0 mm diameter) were impregnated with different dilutions of the reference drugs (gemifloxacin for antibacterial and nystatin for antifungal) and CMEs. Discs were kept in refrigerator prior to use. Extracts and reference drug loaded discs (20 µl) were placed on agar plates. Crude extracts and gemifloxacin loaded nutrient agar plates for determination of antibacterial activity were incubated at 37°C for 24 hours whereas crude extracts and nystatin loaded Sabouraud dextrose agar plates were incubated at 37°C for 7 days to determine antifungal potential. DMSO was used as a negative control in both antibacterial and antifungal activities. Test was performed in triplicates and zone of inhibition was measured with digital vernier calliper after the completion of incubation time period.

Antidiabetic activity

The *in vivo* Antidiabetic study was conducted on Alloxan-induced diabetic rabbits to investigate the hypoglycaemic effects of *D. oleoides and B. baluchistanica* CMEs (Akhtar *et al.,* 1981) with slight modification.

Material and animals

Alloxan monohydrate (98%), α-d-glucose was obtained from Sigma-Aldrich. Abbott Optium Xceed glucometer kit, Carboxymethyl cellulose (CMC) and Healthy adult rabbits (red eyed white chinchilla strain of New Zealand) were obtained from Amson Vaccines & Pharma. Test was performed in the animal house facility of Amson Vaccines & Pharma, Islamabad with the approval of Amson animal ethics committee. International standard guidelines were followed in conducting the studies. Rabbits weighing 1.0-1.5kg were selected for the study. Animals were provided with feed (combination of wheat bran, gram whole black, green fodder and ascorbic acid) and water ad libitum. Rabbits were injected with Alloxan monohydrate (150mg/Kg body weight) intravenously. Blood glucose level was monitored after eight days using glucometer. Rabbits with glucose level between 200-500 mg/dl were considered diabetic for further investigations (Akhtar *et al.,* 1981).

Procedure

Diabetic rabbits were divided into five (I-V) groups with 6 rabbits in each group. Group I was used as a negative control receiving 1% CMC solution. Group II was diabetic control. Rabbits of Group III, IV and V were treated with a single dose of 0.5 g/Kg body weight of standard drug Metformin hydrochloride, *D. oleoides* CME *and B. baluchistanica* CME respectively. Standard drug and extracts were suspended in 1% CMC solution with a final volume of 10ml. 1% CMC and suspended drugs (standard and extracts) were administered orally with a 10 cc plastic syringe attached to stainless steel needle passing through oesophagus to the stomach. Plunger was slowly pushed to administer drugs into the stomach. After administration of samples, rabbits were kept in a wooden rabbit holder, blood glucose levels were checked immediately and after 5, 10 and 24 hours' time intervals. Blood glucose level was determined using Abbott Optium Xceed test strip and glucometer by collecting blood from rabbit ear. Measurements were made twice to ensure uniformity in the glucometer reading. Blood glucose data was analysed using ANOVA Statistical technique in mg/dl (Mean ± SEM).

Antioxidant activity

DPPH radical scavenging assay

2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay was performed to measure the radical scavenging activity (Obied *et al.,* 2005). DPPH solution (0.0032%) was prepared in a mixture of 82 parts of methanol and 18 parts of water. Solutions of test samples (*D. oleoides and B. baluchistanica* CMEs) and control samples (Ascorbic acid and Resveratrol) were prepared in the concentration ranging from 62.5 to 1000 μg/ml. 2.8 ml of DPPH solution and 0.2 ml of each concentration of test and control samples (in triplicate) were mixed separately in glass vial and kept in dark for one hour at room temperature. DPPH solution was used as negative control. Absorbance was measured at 517 nm using UV-Visible spectrophotometer (Shimadzu UV-1800). IC_{50} was calculated using IC_{50} online calculator (AAT bioquest USA).

$$\text{Scavenging Activity (\%)} = (A_C - A_T / A_C) * 100$$

Where,

A_C = absorbance of the DPPH solution.

A_T = absorbance of the test solution.

STATISTICAL ANALYSIS

The measured values displayed are mean ± Standard deviation on triplicate sample and analysed by one-way analysis of variance (ANOVA) by SPSS software. IC_{50} values calculated using IC_{50} online calculator (AAT bioquest USA). A significant P-value was considered when $P < 0.05$.

RESULTS

Cytotoxic activity

Cytotoxic potential of *D. oleoides and B. baluchistanica* CMEs was investigated using MTT assay on normal and cancerous cell lines as shown in table 1. *D. oleoides* exhibited strong percent inhibition against HeLa cells (IC_{50}: 77.87μg/ml) and weaker cytotoxicity against NIH cells (IC_{50}: 117.18μg/ml) whereas *B. baluchistanica* CME showed weak inhibition (IC_{50}: 170.02μg/ml) against HeLa cells and moderate toxicity (IC_{50}: 83.83μg/ml) against 3T3 cells as compared to doxorubicin (IC_{50}: 0.2±0.03 μg/ml) and cyclohexamide (IC_{50}:25.48 μg/ml) standard drugs respectively. Values are presented as mean ± Standard deviation (n = 3), ($P<0.03$).

Antimicrobial activities

Antimicrobial activities of *D. oleoides and B. baluchistanica* CMEs were assessed against different bacterial and fungal strains. Zones of inhibition in millimetre were measured at different concentrations 30, 60 and 90 μg/ml of standard drugs and CMEs (table 2) dissolved in DMSO. Negative control (DMSO) had not shown any inhibitory zones. Maximum zones of inhibition of *B. baluchistanica* CME (14.18, 15.69 and 16.84 mm) and *D. oleoides* CME (11.09, 12.20 and 13.60 mm) were observed against *P. aeruginosa* (Gram-negative bacterium) as compared to standard drug gemifloxacin mesylate (14.40, 23.56 and 33.95 mm) at three different concentrations respectively. Against gram positive strains *B. baluchistanica* CME was found with better inhibitory profile (zone of inhibition of *M. luteus* and *B. subtilis* 16.81, 17.93, 20.22 mm and 12.18, 16.72, 18.37 mm respectively) than *D. oleoides* CME. However inhibition zones of both CMEs were lesser than reference drug (30.65, 33.82, 39.41 mm for *M. luteus*, 24.66, 29.45, 36.95 mm for *B. subtilis*) at three tested concentrations. Superior antifungal potential of *D. oleoides* CME (14.51, 14.72, 15.06 mm) was noted against *S. cerevisiae* as compared to *B. baluchistanica* CME (7.32, 8.64, 8.72 mm) but lesser inhibitory effects than nystatin reference drug (16.97, 17.28, 18.51 mm) at 30, 60 and 90 μg/ml concentrations .Both CMEs had shown no or insignificant antifungal activity against *C. albicans.* Zones of inhibition are presented as mean ± Standard deviation (n = 3), *$P < 0.05$ (significant) while comparing reference drug with extract similarly **$P < 0.05$ (significant) when extracts were compared with each other.

Antidiabetic activity

Alloxan induced rabbits were utilized to determine the antidiabetic prospective of CMEs. Metformin hydrochloride was employed as standard drug. Metformin hydrochloride, *D. oleoides* and *B. baluchistanica* CMEs were administered as dose of 500mg/kg body weight suspended in 10ml of 1% CMC solution to different treatment groups and blood glucose level were monitored at 0, 5, 10 and 24 hrs time intervals. *B. baluchistanica* CME (135.75±0.53mg/dl) produced higher antidiabetic effects compared to *D. oleoides* CME (191.50±0.48 mg/dl) after 24 hrs of dosing. Both extracts were not proved superior antidiabetic agents as compared to metformin hydrochloride (111.58±1.30mg/dl). Antidiabetic potential is summarized in table 3. (Readings were noted using glucometer. Results were displayed as mean ± SEM).

Antioxidant activity

DPPH assay was used to determine the antioxidant activity of *D. oleoides s*and *B. baluchistanica* CMEs. Ascorbic acid and resveratrol were taken as reference drugs. Different concentrations (62.5ppm-1000 ppm) were used and an IC_{50} values were calculated. *D. oleoides and B. baluchistanica* CMEs exhibited higher antioxidant potential (IC_{50}: 52.86 μg/ml and 87.30 μg/ml respectively) compared to resveratrol (IC_{50}: 109.46 μg/ml), however *B. baluchistanica* CME manifested outstanding radical scavenging activity compared to ascorbic acid (IC_{50}: <62.5 μg/ml). Percentage inhibition data is shown in table 4.

Table 1: Cytotoxic activity of *D. oleoides and B. baluchistanica* CMEs

Cell Lines	*D. oleoides* CME Mean ± SEM (% Inhibition)		*B. baluchistanica* CME Mean ± SEM (% Inhibition)	
Concentration (µg/mL)	NIH/3T3	HeLa	NIH/3T3	HeLa
25	14.80 ±0.39	19.55 ±0.67	16.08 ±0.87	19.54 ±1.36
50	38.94 ±0.45	34.62 ±1.28	28.49 ±0.98	26.34±1.98
100	52.06 ±2.83	57.98 ±1.66	47.29 ±2.44	38.88±0.71
200	65.41 ±2.63	69.71 ±3.27	62.54 ±1.79	54.04 ±2.44
IC_{50}	117.18	77.87	83.83	170.02
Doxorubicin IC_{50}: 0.2±0.03 µg/ml, Cyclohexamide IC_{50}:25.48 µg/ml				

IC_{50} is the concentration at which 50% cells are inhibited. SEM = Standard error of mean (n = 3).
D. oleoides is more cytotoxic for cancerous cells and non-toxic for normal cells as compared to *B. baluchistanica.*

Table 2: Antimicrobial activity of *D. oleoides and B. baluchistanica* CMEs

Sample Information		Bacterial strains Zone of Inhibition (mm)						Fungal strains Zone of Inhibition (mm)		
Sample Name	Conc.	Gram-negative				Gram-positive				
	(µg/ml)	*Ec*	*Pa*	*St*	*Sm*	*Ml*	*Bs*	*Sc*	*Cg*	*Ca*
Gemifloxacin	30	35.7	14.4	27.50	18.32	30.65	24.66	—	—	—
	60	35.97	23.56	30.50	23.40	33.82	29.45	—	—	—
	90	40.78	33.95	32.60	27.70	39.41	36.95	—	—	—
Nystatin	30	—	—	—	—	—	—	16.97	18.66	14.45
	60	—	—	—	—	—	—	17.28	18.69	14.77
	90	—	—	—	—	—	—	18.51	18.97	15.28
D. oleoides CME	30	10.17	11.09	9.79	6.55	10.39	8.87	14.51	11.21	—
	60	11.68	12.20	12.10	8.65	12.80	11.62	14.72	11.63	—
	90	12.48*	13.60	13.10*	10.33	13.24	14.53	15.06	11.69*	3.38
B. baluchistanica CME	30	10.23	14.18	12.90	13.35	16.81	12.18	7.32	10.64	—
	60	15.01	15.69	12.96	13.87	18.93	16.72	8.64	10.53	—
	90	15.23	16.84**	16.60*	14.02	20.22**	18.37	8.72*, **	10.96*	—

Ec = Escherichia coli, Pa = Pseudomonas aeruginosa, St = Salmonella typhi, Sm = Stenotropomonas maltophilia, Ml = Micrococcus luteus, Bs = B Bacillus subtilis, Sc = Saccharomyces cerevisiae, Cg = Candida glabrata, Ca = Candida albicans
* = P<0.05, reference drug against extracts, ** = P<0.05, significance among extracts

Table 3: Antidiabetic activity of *D. oleoides and B. baluchistanica* CMEs

Group	Treatment	0 hrs.	5 hrs.	10 hrs.	24 hrs.
I	Negative control	84.42 ± 1.01	85.67 ± 0.77	89.08 ± 0.53	87.83 ± 0.58
II	Diabetic control	321.00 ± 1.35	331.00 ± 1.89	356.33 ± 1.15	422.33 ± 0.67
III	Metformin HCl	304.67 ± 1.06	199.42 ± 1.68	150.33 ± 1.54	111.58 ± 1.30
IV	*D. oleoides*	281.58 ± 1.59	247.92 ± 1.30	219.00 ± 0.87	191.50 ± 0.48
V	*B. baluchistanica*	292.83 ± 0.67	231.25 ± 1.29	169.92 ± 0.91	135.75 ± 0.53*

Table 4: Antioxidant activity of *D. oleoides and B. baluchistanica* CMEs

Antioxidant Activity (% inhibition)				
Concentration (ppm)	Ascorbic Acid	Resveratrol	*D. oleoides CME*	*B. baluchistanica CME*
1000	NA	NA	93.62	91.83
500	96.73	95.84	91.84	89.76
250	97.33	59.68	84.42	83.67
125	97.18	51.82	58.01	77.74
62.5	97.03	35.05	34.86	52.07
IC_{50} (µg/ml)	<62.5	109.46	87.3	52.86*

% Inhibition calculated as mean ± Standard deviation (n = 3), P < 0.05 (significant*)

DISCUSSION

Anticancer, antimicrobial, antioxidant and antidiabetic activities of *D. oleoides* and *B. baluchistanica* CMEs were investigated in the present research. Medicinal plants are widely explored to discover new lead molecules with diversified structure and mechanism to treat infectious ailments (Rojas *et al.,* 2003). Current studies ascertained the cytotoxic potential of *D. oleoides* and *B. baluchistanica* CMEs on NIH and HeLa cells using MTT assay. Plant derived chemo protective agent must have less than 100µg/ml IC_{50} value to be potent cytotoxic agent (Prayong *et al.,* 2008). Previously *in vitro* brine-shrimp cytotoxicity assay (no cell line) was reported for *B. baluchistanica* (Baloch *et al.,* 2013), however in this research anticancer activity against HeLa and NIH Cell lines is being reported for the first time. *B. baluchistanica* CME showed more toxicity against normal NIH cells (IC_{50}: 83.83µg/ml) as compared to *D. oleoides* CME (IC_{50}: 117.18µg/ml). In case of HeLa cells *D. oleoides* CME (IC_{50}: 77.87µg/ml) was more toxic than *B. baluchistanica* CME (IC_{50}: 170.02µg/ml), although both CMEs had not displayed a significant cytotoxic behaviour compared to standard drugs cyclohexamide and doxorubicin. Growth inhibition of HeLa cells may be attributed to the presence of flavonoids, terpenoids, coumarins and phenolic compounds. Latest studies reveals that coumarins obtained from *D. oleoides* exhibited cytotoxic potential against different cell lines like HBL-100, HT-29, HCT-116 and A549 (Dar *et al.,* 2019). Diterpenes isolated from Thymelaeaceae were also proven as potent anticancer compounds against human leukemic, liver A549 and lung Hep2 cancer cell lines (Carswell *et al.,* 1975; Jiang *et al.,* 2015). Current study suggested *D. oleoides* as a potential source of natural cytotoxic compounds.

In case of antimicrobial activity both Gram-positive and Gram-negative bacteria were moderately inhibited by both plants CMEs but gram-positive bacteria were more susceptible to *B. baluchistanica* CME. The difference in antibacterial potential may be attributed to strains morphology and presence of variable constituents. Among Gram-negative strains, *B. baluchistanica* significantly inhibited *P. aeruginosa* as compared to gemifloxacin standard drug, similarly *P. aeruginosa* was most sensitive strain to *D. oleoides* CME (table 2). Literature reveals that phenolic, tannins and flavonoids demonstrated significant activity against Gram-negative strains (Ahmad and Beg, 2001; Rios and Recio, 2005). Beside gram-positive strains *M. luteus* and *B. subtilis* were sensitive to *B. baluchistanica* CME and *D. oleoides* CME respectively.

Among fungal strains *S. cerevisiae* was more but *C. albicans* was least sensitive to *D. oleoides* CME. The existence of various flavonoids have been reported in *D. oleoides* (Riaz *et al.,* 2016) that form complexes with extracellular proteins of fungal cell wall thereby disrupting its integrity and making them a suitable antifungal agent (Arif *et al.,* 2009). On the other hand *C. glabrata* was most affected and *C. albicans* remained resistant to *B. baluchistanica* CME. Alkaloids like berberine and berbamine of berberis species have been shown to exert its antifungal action by disrupting the mitochondrial & plasma membranes and also obliterate DNA causing cell death (da Silva *et al.,* 2016).

A considerable drop in blood glucose levels were observed in diabetic rabbits. *B. baluchistanica* CME produced superior hypoglycaemic effect to that of *D. oleoides* CME though not equivalent to standard Metformin Hydrochloride. Previous studies postulate that the blood glucose lowering effect of *B. baluchistanica* CME may be associated with alkaloids and glycoside constituents. Berberine and oleanolic acid (a triterpenoid and aglycone part of glycoside) possess significant *in vivo* hypoglycaemic property (Gulfraz *et al.,* 2008; Wang *et al.,* 2011). Berberine and oleanolic acid isolated from *B. baluchistanica* (Pervez *et al.,* 2019) might be supporting glucose lowering behaviour. Hypoglycaemic effects of *D. oleoides* CME was reported for the first time in this report but the results were not promising .Further exploration is required to discover the unique biologically active compound responsible for producing hypoglycaemic behaviour of *B. baluchistanica* CME.

Antioxidant mechanism of different molecules depends either on hydrogen atom transfer (HAT) or single electron transfer (SET) and ability to chelate metals. HAT is a free radical scavenging that acts by breaking the chain reaction. DPPH was used to determine radical scavenging potential of *D. oleoides* and *B. baluchistanica* CMEs. The methodology for using DPPH was to measure the reduction of free radicals by antioxidant compounds. The remaining quantities of DPPH when initial values were reduced to 50% were determined and calculated as IC_{50} value. Low IC_{50} values confirm high antioxidant potential. *B. baluchistanica* CME indicated more radical scavenging effect as compared to *D. oleoides* CME and resveratrol reference drug. Literature reveals that berberine (alkaloid) obtained from berberis species and berberisinol (flavones) from *B. baluchistanica* exhibited antioxidant potential (Pervez *et al.,* 2019; Shirwaikar *et al.,* 2006). Berberine is also present in *B. baluchistanica* (Pervez *et al.,* 2019) therefore, berberine and berberisinol having hydrogen donor capacity might be a contributing factor for scavenging aptitude of *B. baluchistanica* CME. *D. oleoides* CME also indicated relatively better scavenging of free radical compared to resveratrol. Polyphenols and flavonoids are vital compounds for antioxidant activity of plants extract due to their reactive oxygen quenching, redox, hydrogen donating and metal chelating properties (Rice-evans *et al.,* 1995). Radical scavenging activity of

D. oleoides CME can be attributed to the occurrence of flavonoids and polyphenols (Riaz *et al.,* 2016). However, antioxidant potential of both CMEs was less significant than ascorbic acid.

CONCLUSION

The data presented in this paper illustrates comparative studies of biological activities showed by methanolic extract obtained from roots of *D. oleoides* and stem of *B. baluchistanica. D. oleoides* showed substantial cytotoxic behaviour against HeLa cancerous cell lines, bactericidal against *P. aeruginosa* and fungicidal against *S. cerevisiae* and *C. glabrata*. *B. baluchistanica* exhibited significant antioxidant potential, pronounced antibacterial activity against *P. aeruginosa, bacillus subtilis* and *M. luteus* bacterial strains and substantial in vivo hypoglycaemic properties against Alloxan induced diabetic rabbits. *D. oleoides* and *B. baluchistanica* be worthy for further scientific investigation to isolate and identify potentially biologically active lead molecule.

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