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Antioxidant, Cytotoxicity and Antitumor of *Bergia suffruticosa* (whole plant)

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Abstract- *Bergia suffruticosa* (Elatinaceae) is a plant that is collected from western Sudan. It is popularly used traditionally in the treatment as wounds healer and osteitis. This research aims to screen the cytotoxicity as well as antioxidants and the impact as antitumor of *Bergia suffruticosa* metabolites. Metabolites were extracted using different organic solvents to increasing polarity. The antioxidant was assessed using free radical scavenging activity DPPH. The petroleum ether extract was revealed the strongest antioxidant with IC_{50} 89±0.01µg/ml, while the aqueous extract was revealed no antioxidant activity. The cytotoxicity was evaluated using the Brine shrimp lethality assay. Petroleum ether metabolites revealed high cytotoxicity with LD_{50} 0.04ppm while the other metabolites were exhibited no cytotoxicity. The antitumor activity was assessed using SRB assay against tumor cell lines, breast carcinoma cell line (MCF7), Prostate carcinoma cell line (PC3), colon carcinoma cell line (HCT116). All metabolites were revealed activity against all cancer cells tested. The highest activity against breast cancer was exhibited by ethyl acetate with IC_{50} 17 µg/ml. the petroleum ether extract was revealed the highest activity against prostate cancer cells with IC_{50} 17.9 µg/ml.

Keywords- *Bergia suffruticosa*; Elatinaceae; Antioxidant; Cytotoxicity; Antitumor.

I. INTRODUCTION

Reactive oxygen and free radicals have an important role to play in the initiation and development of numerous illnesses. The antioxidant compounds are expected to be useful for the treatment of such diseases. Therefore, the demand has increased for such a remedy for finding new antioxidants to meet the needs of the pharmaceutical industry [1]. To reduce the potential for oxidative deterioration, artificial antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been added to foods. These compounds are presumed to be carcinogenic and cause liver damage [2]. Therefore, there is great interest in finding new and safe antioxidants from natural sources. Breast cancer is the second most common cancer in the United States, with approximately 230,000 new cases discovered annually (www.cancer.org). Several breast cancer cell lines exist for research studies, including the well-characterized MCF-7 and MDA MB-231 cells. [3]. The extractive solvent is frequently used to isolate the antioxidants and all extraction results, phenol content and antioxidant activity of the extracts are highly dependent on the solvent, as the different antioxidative potentials of compounds with different polarity [2]. *Bergia suffruticosa* belongs to the family Elatinaceae, growth in a different area in Sudan. *Bergia suffruticosa* phytoconstituents are phenols, alkaloids, carbohydrates, and no saponins [1]. Traditionally *Bergia suffruticosa* was used as a wound healer [4], to treat urinary tract infection and as antihypertensive [5], and in treatment of osteitis [6]. The present study has assessed the antioxidant, cytotoxicity and antitumor activity of *Bergia suffruticosa* raw extracts.

II. MATERIALS AND METHODS

Materials

All solvents as Petroleum ether, 2,2-diphenyl-1-hydroxyl (DPPH), trichloroacetic acid were obtained from Sigma Aldrich (Germany). Ferric chloride, dimethyl sulfoxide (DMSO) was purchased from Riedel-de Haën (Germany). All other chemicals were of analytical grade.

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Cell line as breast carcinoma cell line (MCF7), Prostate carcinoma cell line (PC3), colon carcinoma cell line (HCT116) were obtained frozen in liquid nitrogen (-180°C)from the American Type culture Collection .The tumor cell lines were maintained in the National cancer Institute ,Cairo ,Egypt ,by serial sub –culturing.

Plant material preparation

Bergia suffruticosa (whole plant) was collected in March from Elobaied North Kordofan. The plant was authenticated by the Medicinal and Aromatic plants Research Institute, Khartoum, Sudan. The collected plant was dried under the shade and reduced to a coarse and used for extraction.

Preparation plant extracts

A weight (75g) the coarsely powdered shade-dried plant sample was extracted in a soxhlet extractor successively with organic solvents of increasing polarity petroleum ether, ethyl acetate, and ethanol. On the other hand, 75g also were macerated with 70% methanol (250ml) and another equal weight was decocted with 250ml distilled water. Each extract was filtrated and concentrated using a rotator vacuum evaporator to a constant weight. The dry extract was kept at +4ºC until tested. Before use, the petroleum ether and ethyl acetate extracts were dissolved in dimethylsulfoxide (DMSO), ethanol, 70%methanol, and aqueous extracts were dissolved in methanol [2].

DPPH free radical scavenging activity

The free radical scavenging activity was followed by the DPPH method. 0.1 mM solution of DPPH was prepared in methanol and 1.0 ml of this solution was added to 3.0 ml of extract solution at different concentrations (50, 100, 200 µg/ml) and incubated for 30 minutes after that, the absorbance was measured at 517 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations (50, 100, and 200 µg/ml) was used as standard. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH Scavenged (\%)} = [(\text{A control} - \text{A test}) / \text{A control}] \times 100$$

Where a control is the absorbance of the control reaction and A test is the absorbance in the presence of the sample of the extracts. The antioxidant activity was expressed as IC50 and compared with standard. the extracts had significant scavenging effects on the DPPH radical which was increasing with the increase in the concentration of the sample from 10-200 µg/mL [7].

Brine Shrimp Lethality assay

The brine shrimp lethality assay was carried out with slight modification. A 10,000 mg/L stock solution in ethanol was prepared. Serial dilutions were prepared by measuring an appropriate volume of 500.0, 250.0, 50.0, 25.0, 5.0 and 2.5 µL of the stock solution placed into separate test tubes and marked up to 5.00 mL to give 1000, 500, 100, 50, 10 and 5 mg/L, respectively. The solution was purged with N2 gas to remove the solvent. Minimal amount of Dimethyl Sulfoxide (DMSO) corresponding to 50.00, 25.00, 5.00, 2.50, 0.50 and 0.25 µL for 1000, 500, 100, 50, 10 and 5 mg/L, respectively, were added as liquid surfactant into each test tube. The mixture was then vortexes to ensure homogeneity. Ten brine shrimp nauplii were transferred from the hatching chamber into each test tube. Sterilized seawater was added up to the 5 mL mark and the test tubes were incubated under illumination for 24 hrs. Negative and positive controls were run using sterilized seawater with DMSO and potassium dichromate, respectively. The number of surviving shrimps were counted and recorded after 24 hrs. The percentage mortality of nauplii was then calculated. For sample test solutions giving 0% and 100% mortalities, necessary corrections were done. Using the Probity Table of Finney, the corrected percentage mortality values were transformed to probity values. The best-fitting straight curve was then drawn with the logarithm of the concentration values as the abscissa and the probity values as the ordinate. The log10 concentration value that corresponds to the probity point of 5.00 from the curve was recorded and converted to its antilog value to give the LC_{50}. LC_{50} of less than 200 mg/L was considered cytotoxic Analysis was carried out in five replicates. The result was then reported as a mean of the analysis [8].

Antitumor activity

II- Sulphorhodamine-B (SRB) assay of cytotoxic activity

Cells were used when 90 % of confluence was reached in T25 flasks. Adherent cell lines were harvested with 0.025 % trypsin. Viability was determined by trypan blue exclusion using the inverted microscope (Olympus 1x70, Tokyo, Japan). Cells were seeded in 96-well microtiter plates at a concentration of 5x104-105cell/well in a fresh medium for 24 hrs to attach to the plates. Then, cells with the extract at the appropriate concentration, and completed to a total of 200 µl volume/well using fresh medium, incubated for 24, 48 and 72 hrs. Control cells were treated with vehicle alone. 4 wells were used for each extract concentration.

The cells were fixed with 50 µl cold 50% trichloroacetic acid for 1 hr at 4 ºC. The Wells were washed 5 times with distilled water and stained for 30 min at room temperature with 50 µl 0.4 % SRB dissolved in 1 % acetic acid and washed 4 times with

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1 % acetic acid. The plates were air-dried and the dye was solubilized with 100 µl/well of 10 mM tris base (ph 10.5) for 5 min on a shaker (Orbital shaker OS 20, Boeco, Germany) at 1600rpm. The optical density (O.D.) of each well was measured spectrophotometrically at 564nm with an ELIZA microplate reader (Meter tech. Σ 960, U.S.A.). The mean background absorbance was automatically subtracted and means values of each drug concentration were calculated [8]–[10].

III. RESULTS

Antioxidant activity

Antioxidant scavenging property of the extract was detected using DPPH activity. By increasing the concentration of extract there is decrease the DPPH radicals by IC_{50} (Table: 1).

Table 1: Antioxidant activity of *Bergia suffruticosa* whole plant determined by DPPH

Concentration (µg)	%RSA ± SD (DPPH)					
	Standard (ascorbic acid)	Plant Extract				
		Petroleum ether	Ethyl acetate	Ethanol	70% Methanol	Aqueous
100	98.80+ 0.11	88 ± 0.25	55 ± 0.14	82 ± 0.00	85 ±0.12	12 ± 0.04
200	99.76+0.11	89±0.01	58±0.07	83±0.02	86±0.00	12±0.04

Brine shrimp lethality assay

Percent lethality and the LC_{50} values of the brine shrimp obtained after chronic exposure to varying concentrations of the whole plant extracts of *Bergia suffruticosa* (Table: 2).

Table 2: Cytotoxicity of *Bergia suffruticosa* whole plant determined by Brine shrimp Lethality assay after 24hrs

Plant extract	Concentration of extract (ppm)	No. of Brine Shrimps	No. of Brine Shrimps death	Brine Shrimps Mortality* (%)	LD50 (µg/ml)	Inference**
Petroleum ether	10	30	27	90	0.04	Cytotoxic
	100	30	29	97		
	1000	30	30	100		
Ethyl acetate	10	30	5	16	321.1	Non- Cytotoxic
	100	30	5	16		
	1000	30	30	100		
Ethanol	10	30	10	30	226.7	Non- Cytotoxic
	100	30	2	60		
	1000	30	25	83		
70%Methanol	10	30	9	30	112.0	Cytotoxic
	100	30	6	20		
	1000	30	28	93		
water	10	30	1	03	220.2	Non- Cytotoxic
	100	30	7	23		
	1000	30	30	100		

* Each sample was analyzed five times

**LC50 < 200 mg/L is cytotoxic [11].

Antitumor activity using II- Sulphorhodamine-B (SRB) assay

The sensitivity of the human tumor cell lines to thymoquinone was determined by the SRB assay. SRB is a bright pink aminoxanthrene dye with two sulphonic groups. It is a protein stain that binds to the amino groups of intracellular proteins under mildly acidic conditions to provide a sensitive index of cellular protein content. The optical density (O.D.) was measured spectrophotometrically at 564nm with an ELIZA microplate reader (Meter tech. Σ 960, U.S.A.). The mean background absorbance was automatically subtracted and means values of each drug concentration were tabulated (Table 3).

Table 3: Cytotoxicity of *Bergia suffruticosa* Whole plant determined by Brine shrimp Lethality assay after 24hrs

Plant extract	IC_{50} (µg/ml)		
	MCF7	PC3	HCT
Petroleum ether	20	17.9	24.2
Ethyl acetate	17	21.7	35.2
70% Methanol	21.5	21.8	35.2
Water	20.8	37	Nd

Key: Nd= not detected,

IV. DISCUSSION

The present study was attempted to assess the antioxidant capacity, cytotoxicity and antitumor activity of *Bergia suffruticosa* whole plant extracts. 1, 1- Diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical which can be effectively scavenged by the antioxidants in the substrate and convert it into 1, 1-Diphenyl-2-picrylhydrazine [12]. The petroleum ether extract was revealed the highest scavenging power compared with the scavenging power of ascorbic acid at concentration 10 µg [6]. Aqueous methanol (70%) extract which is obtained by the maceration process was revealed higher scavenging power compared with ethanol extract obtained by the soxhlet method. Although alcohol extracts the polar phenol metabolites the result reflects variation in the antioxidant effect, this may be due to the effect of heat in the stability of the scavenging metabolites[7][2][1]. Brine shrimps with the different extracts at concentrations 1000 ppm were exhibited 100% mortality. Petroleum ether extract and 70% methanol were revealed potential cytotoxic properties according to [11]. This result was agree with [10], [11], [13]. The effect of processing in reducing the toxicity was observed in the aqueous decocted extract [5][14].

According to SRB assay, all metabolites were revealed activity against all cancer cells tested. The highest activity against breast cancer was exhibited by ethyl acetate with IC_{50} 17 µg/ml, while the petroleum ether extract was revealed the highest activity against prostate cancer cells with IC_{50} 17.9 µg/ml. this result is matched with the result of cytotoxicity as table 2. This result was supported with [8], [9], [14]–[18][19].

V. CONCLUSION

Bergia suffruticosa crude extracts, irrespective of solvent type, exhibited antioxidant activity. The petroleum ether extract was revealed the highest antioxidant effect compare to the other extract. On the other hand, petroleum ether exhibited a cytotoxic effect and inhibit the growth of breast cancer cells.

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