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Phytochemical screening, Anticancer and cytotoxicity of *Pulicaria crispa* (whole plant)

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Abstract- *Pulicaria crispa* (**Asteraceae**) is an indigenous plant in Western Sudan. All parts of the plant are traditionally used to heal various diseases, especially those related to the respiratory system. The aim of this study was the chemical screening of metabolites extracted from the plant. Besides, test their toxicity, and their inhibition effect on cancer cells. The metabolites were completely extracted from the plant using three methods: Soxhlet with petroleum ether, ethyl acetate, and ethanol. Maceration with 70% methanol, and decoction with distilled water. The results of chemical screening of all extracts obtained, except the petroleum ether extract, showed the presence of alkaloids, flavonoids, phenols, carbohydrates, terpenes, proteins, and lipids. The Soxhlet petroleum ether extract showed no alkaloids, carbohydrates, and proteins. The cytotoxicity of plant metabolites was tested using brine shrimp lethality assay. The results showed that Soxhlet extracts revealed significant toxicity ($P < 0.05$) to brine shrimp larvae compared to other extracts. Anticancer was investigated by sulforodamine B (SRB) screening against three lines of cancer cells: breast cancer cell line (MCF7), colon cancer cell line (PC3), and prostate cancer cell line (HCT116). The results reflected that Soxhlet extracts had a significant effect on inhibiting the growth of breast and colon cancer cells besides ethyl acetate extract was also inhibit the growth of prostate cancer cells. The maceration and decoction extracts were revealed no anticancer activity against all cancer cells tested.

Keywords -*Pulicaria crispa*, Asteraceae, Cytotoxicity, Sulphorhodamine-B, Breast cancer cell, Colon cancer cell, Prostate cancer cell, Brine Shrimps.

I. INTRODUCTION

Nature has been an excellent source of therapeutic agents since immemorial time [1]. Traditional and conventional medicines are mostly based on numerous natural resources, including plants that are being used as a primary source in folkloric medicine [2]. Before the discovery of chemical and synthetic compounds, these plants played a significant role as a traditional medicine for curing various diseases throughout the world [3]. *Pulicaria crispa* belongs to the most important aromatic family **Asteraceae** [4]. *Pulicaria* comprises approximately 100 species which are widely distributed from Asia into North Africa and Europe, particularly around the Mediterranean basin [5]. *Pulicaria crispa* is found in Elobaied, North Kordofan, Sudan [6]. *Pulicaria crispa* traditionally used in the treatment of a variety of illness such as cold, flu [4], heart disfunction, and inflammations [7], intestinal disorders [8], antibacterial, antihistamine, antifungal, and insecticide [9], anticancer properties, as well as inhibitory effects on nitric oxide production [10]. *Pulicaria crispa* metabolites as carbohydrates, terpenes, alkaloids, flavonoids, and phytosterols [9,10]. This research aims to screen the phytoconstituents and to test the cytotoxicity of *Pulicaria crispa* extracts to improve or reject the traditional uses and aid in finding new natural sources as antitumor.

II. MATERIALS AND METHODS

Collection, Identification, and Preparation of *Pulicaria crispa*

Pulicaria crispa was collected from (Elobaied) North Kordofan, Sudan in 2013 and authenticated by Medicinal and Aromatic plants & Traditional Medicine Research Institute, National Center for Research, Khartoum, Sudan. All solvents, chemical used were of analytical grade. The clean shade dry plant material was ground using an electric house-hold spice grinder.

Preparation of *Pulicaria crispa* whole plant extract

The metabolites of the prepared plant material were extracted using three different methods

Continuous extraction method

A weight of powdered plant material (75gms) was extracted using the soxhlet apparatus with petroleum ether, ethyl acetate, 70% methanol, and finally with distilled water. The filtered extract was dried to constant temperature using a rotary evaporator and kept at 4ºC until used for further investigation [6].

Maceration method

Weight of the powdered plant material (100gm) was macerated with a volume of organic solvent (750ml), 70% methanol and water, respectively, at room temperature for

42 hours, then filtrated and dried to constant weight and refrigerated at 4ºC until used for further investigation [11].

Decoction method

This method is prepared as the traditional preparation. Weight of the powdered plant material (50gm) was decocted with distilled water for 15 min, then cools to room temperature and filtered. The filtrate was dried to constant weight and kept at 4ºC until used for further investigation [12].

Phytochemical screening

The general Phytochemical screening was carried as described [4] to test the main Phytochemical groups: phenols, alkaloids, terpenes, carbohydrates, and proteins, and lipids

Cytotoxicity of *Pulicaria crispa* whole-plant extracts

Brine shrimps lethality assay

Brine shrimps lethality assay was used to test the cytotoxicity of the prepared extracts at different concentrations (10, 100, and 1000 µg/mL) against *Artemia salina* larvae. The number of survivors was counted and recorded and compared with reference cytotoxic drug (Etoposide) served as a positive control. The data were processed using a Finney computer program as described by [13] and the LD50 values were obtained at 95 % confidence intervals.

Anticancer Screening of *Pulicaria crispa* whole-plant extracts

II- Sulphorhodamine-B (SRB) assay

Cells were used when 90% of flounce was achieved in T25 flasks. Attached cell lines were harvested with 0.025 % trypsin. The viability was determined by trypan blue elimination with the inverted microscope (Olympus 1x70, Tokyo, Japan). Cells were cultured in 96-well microtiter plates at a concentration of 5x104-105 cells/well in the freshly prepared medium for 24 hours to attach to the plates. The cells were then seeded with the extract at the appropriate concentration and using the freshly prepared medium to a total of 200 µl volume/well and were incubated for 24, 48, and 72 hours. The control cells were treated with solvent only. For each extract concentration, 4 wells were used.

The cells were fixed with 50 µl cold 50% trichloroacetic acid for 1 hour at 4 ºC. The wells were well washed 5 times with distilled water and stained with 50 µl 0.4 % SRB dissolved in 1 % acetic acid for 30 minutes at room temperature and washed 4 times with 1 % acetic acid. The plates were air-dried and the dye was diluted with 100 µl/well 10 mM Tris-Base (ph 10.5) for 5 min on a vibration device (orbital shaker OS 20, Boeco, Germany) at 1600 rpm. The optical density of each well was measured spectrophotometrically at 564 nm with an ELIZA microplate reader (meter tech. Σ 960, U.S.A.). The mean values of background absorbance were automatically subtracted and the mean values of the individual drug concentrations were calculated [6].

III. RESULTS

The Phytochemical screening of the *Pulicaria crispa* whole plant extract represents the presence of all compounds tested in Table 1. The petroleum ether was exhibited the detection of all metabolites except alkaloids, carbohydrates, and proteins.

Table 1: Phytochemical screening of the *Pulicaria crispa* whole plant extract

Phytochemical group	Extraction method						
	Soxhlet				Maceration		Decoction
	***Pulicaria crispa* whole plant extract**						
	Petroleum ether	Ethyl acetate	Methanol 70%	Aqueous	Methanol 70%	Aqueous	Aqueous
Alkaloids	-	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+
phenols	+	+	+	+	+	+	+
Carbohydrates	-	+	+	+	+	+	+
Triterpenes	+	+	+	+	+	+	+
Proteins	-	+	+	+	+	+	+
Lipids	+	+	+	+	+	+	+

The cytotoxicity of using Brine shrimps lethality

All extracts obtained by the soxhlet were revealed significant cytotoxicity ($P < 0.05$) compared with the positive control used. The brine shrimps larvae were survived against all other extracts obtained by maceration and decoction methods Table 2.

Table 2: Cytotoxicity of the *Pulicaria crispa* whole plant extract using Brine Shrimps

Extract concentration (µg/ml)	Extraction method						
	Soxhlet				Maceration		Decoction
	Pulicaria crispa whole plant extract						
	Petroleum ether	Ethyl acetate	Methanol 70%	Aqueous	Methanol 70%	Aqueous	Aqueous
	LD50 (ppm)						
10	37.9	27.1	651.1	No death	No death	No death	No death
100	37.1	25.1	624.2	No death	No death	No death	No death
1000	18.7	10.7	620.8	No death	No death	No death	No death

On the other hand, the soxhlet extracts were exhibited cytotoxicity against the breast and colon cancer cells while the methanol 70% was exhibited also cytotoxicity against the prostate cancer cell. All other extracts obtained by the maceration and decoction methods were revealed no cytotoxicity against all cancer cells tested (Table 3)

Table 3: Anticancer screening of the *Pulicaria crispa* whole plant extract

Carcinoma cell line	Extraction method						
	Soxhlet				Maceration		Decoction
	Pulicaria crispa whole plant extract						
	Petroleum ether	Ethyl acetate	Methanol 70%	Aqueous	Methanol 70%	Aqueous	Aqueous
	IC50 (µg/ml)						
Breast	18.8	21.7	23.0	<50.5	<50.5	<50.5	<50.5
Colon	22.7	20.3	40.9	<50.5	<50.5	<50.5	<50.5
Prostate	<50.5	<50.5	39.0	<50.5	<50.5	<50.5	<50.5

IV. DISCUSSION

Pulicaria crispa is used widely in traditional medicine in Sudan. The use of this plant traditionally usually a macerated extract, or in a decoction tea. The results have reflected the detection of all primary and secondary metabolites tested and agree with [4], [8],[10], [14]. The extracts obtained by the soxhlet was represented cytotoxicity against the brine shrimps larvae this is agreed with [7]–[9], [12], [15]. The macerated and decocted extracts were exhibited no cytotoxicity this agree with [9], [14], [16],[18]. This result supported the safety of the traditional used as macerated and decocted preparation. The anticancer activity of the soxhlet extracts was supported [19]–[22]. In general, the processing affects the cytotoxicity as reported in review [9], [17].

V. CONCLUSION AND RECOMMENDATIONS

Pulicaria crispa is abundant in many secondary metabolites, especially flavonoids, which are present in a large percentage. This supports their manifold applications in Sudanese folk medicine for the cure of many diseases.

The study revealed that this plant is of high importance and rich in metabolites with therapeutic effects, but it needs further investigation, e.g. to isolate and identify metabolites and to study the effectiveness of these metabolites, the therapeutic mechanism, the effective and toxic dose, etc.

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