

Cytotoxic and Antimicrobial Activities of Some Compositae Plants Growing in Taif Area, Saudi Arabia

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ABSTRACT: Family Compositae (Asteraceae) is one of the largest family of flowering plants. In Taif area KSA, there are 28 plants belonging to 21 genera. In this study, the methanolic extracts of some Compositae plants were tested for their in-vitro anti-microbial activity against different strains of bacteria and fungi, and in-vitro cytotoxic screening on different human cancer cell lines. The plants of this study are *Psiadia punctulata*, *Osteospermum vaillantii*, *Echinops spinosissimus*, *Euryops arabicus*, *Launaea mucronata*, *Verbesina encelioides*, *Pulicaria crispa* and *Onopordum heteracanthum*. The antibacterial assay was performed by disc diffusion method and the cytotoxic activity was achieved against breast (MCF7), ovarian (A2780) and cervical (HeLa) cancer cell lines. The obtained results showed that, *Pulicaria crispa*, *Verbesina encelioides* and *Psiadia punctulata* have the most remarkable antibacterial activity while the other plant extracts were less active. Only the extracts of *Pulicaria crispa* and *Psiadia punctulata* exhibited antifungal activity. The cytotoxicity study revealed that *Osteospermum vaillantii* and *Onopordum heteracanthum* have a promising cytotoxicity against different cancer cell lines that somehow show some selectivity toward certain cancer cell types.

KEYWORDS: Compositae plants; Antimicrobial, Cytotoxic Activities.

I. INTRODUCTION

Taif is a city in Makkah Province of Saudi Arabia at an elevation of 2500 m above sea-level [1]. It is characterized by many types of mountains, plateaus and valleys, which support the growth of a number of fruits, vegetables and wild plants. The wild plants are about 181 species, belonging to 137 genera, under 56 families [2], each environment has its own species of plant. Family Compositae (Asteraceae) is one of the largest family of flowering plants [3] spread across 1620 genera. In Taif area, there are 28 plants, belonging to 21 genera [2]. The study includes: *Psiadia punctulata*, *Osteospermum vaillantii*, *Echinops spinosissimus*, *Euryops arabicus*, *Launaea mucronata*, *Verbesina encelioides*, *Pulicaria crispa* and *Onopordum heteracanthum*.

Psiadia punctulata (DC.) Vatke is a branched shrub ranging up to 6 ft. or more. The leaves have a shiny look and develop a sticky feel soon after they are plucked; this is due to the presence of leaf exudate, which is found as 24% dry leaf weight [4,5]. It is common to the Arab Peninsula in the folk medicine and used by Bedouins in casts for broken bones [6]; the warm extract of the leaves and stems in foot injuries suffered by villagers who do not wear shoes. In east African (Kenya), leaf decoction finds a variety of ethnobotanical uses, including treatment of cold, fever, abdominal pains and for removal of ectoparasites from cattle [5]. Traditionally used to relieve pain including abdominal pain [7]. The biological and phytochemical studies include the cytotoxic activity against human cancer cell lines: breast cancer, hepatocellular carcinoma, cervix cancer, urinary bladder carcinoma and nasopharynx human carcinoma [8-10]. The leaf exudate and one isolated diterpene showed analgesic activity in mouse tail flick experiment when compared with aspirin [7]. Also, the antimicrobial and antioxidant activities were studied [9]. In addition, the plant extracts were tested for *in-vitro* antileishmanial activity in cell free cultures and in infected macrophages and the inhibitory concentrations and levels of cytotoxicity were determined [11]. The plant was also tested against *Plasmodium falciparum*,

Trypanosoma brucei brucei, *Trypanosoma cruzi* and *Leishmania infantum*, as well as toxicity against MRC-5 fibroblast cells [12,13]. The bioactivity against the larval and adult stages of *Anopheles arabiensis* in addition to repellent effect were studied [14,15]. The phytochemical studies on the plant resulted in the isolation of diterpenes [5,16-18], flavonoids [6,7,17-20] and phenylpropanoids [19].

Osteospermum vaillantii Decne is a perennial erect, slender-stemmed herb, with simple, entire or few-toothed, half-clasping, oblong-lanceolate leaves [2]. According to the available literatures, it was reported the isolation and structure elucidation of saponins [21,22].

Echinops spinosissimus Turrais an erect branching, perennial spiny-leaved herb [2]. In North-African countries, are commonly used in the form of crude extracts, infusions or plasters for reduction of inflammations and in traditional medicines as a diuretic and as an antifungal [23]. The biological studies include the antimicrobial activity [24,25]; the anti-inflammatory activity [23]; the antioxidant and *in-vitro* cytotoxic effect [24] and *in-vitro* antiprotozoal activity and cytotoxicity on a mammalian kidney fibroblast (Vero) cell line [26]. The plant spp. are rich resource for sesquiterpene lactones, triterpenes, and benzothiophene glycosides in addition to guaianolides and eudesmanolides [23].

Euryops arabicus Steud is a perennial shrub with dense, narrow, sessile, alternate, linear leaves, crowded at the end of branches [2]. The leaves and stems are used for treating wounds [2,27]. The antioxidant effect of the extract and the essential oil was studied [24,28] and the cytotoxic activity of the methanolic extract was determined [8]. Furthermore, the plant extracts were tested for their antimicrobial activity [9,29]. The essential oil of the aerial parts was studied by GC and GC/MS and found that, it contains high percentage of oxygenated sesquiterpenes (39.9%), sesquiterpene hydrocarbons (24.1%) and less percentage of caryophyllene oxide (8.6%), T-cadinol (7.0%), spathulenol (5.2%), (E)- β -caryophyllene (6.0%) and 2-epi-(E)- β -caryophyllene (6.0%) [28,29]. The aerial parts afforded furoeremophilanes, eremophilanolides, two seco-derivatives and a rearranged spiro lactone in addition to two quercetin derivatives and an unusual diester of glucose [30].

Launaea mucronata (Forssk.) Muschl. has a dichotomously-branched blue stem and grey-green, long leaves [2] and it is used as galactagogue and its decoction is administered in constipation [31]. Reviewing the current literature, nothing could be traced about the phytochemical or biological studies of this plant.

Verbesina encelioides (Cav) Benth. and Hook. f. ex A. Gray is commonly known as golden crown beard. The plant is an upright to sprawling annual commonly of 30- 50 cm height [32]. The reported biological activities of the plant includes the cytotoxic activity of the methanolic extract [8]; the nematocidal activity against the root-knot nematode *Meloidogyne javanica* [33]; the hypolipidemic effect of the ethanolic and aqueous extracts of the roots [34]; the antiprotozoal, cytotoxic and antileishmanial activity [12]. The other biological activities are antibacterial, antifungal, antiviral, hypoglycemic and anti-implantation [32,35]. The phytoconstituents include terpenoids, flavonoid glycosides, benzyl-2, 6-dimethoxy benzoate, bornyl ferulate and the toxic galegine [32].

Pulicaria crispa (L.) C. A. Mey. is a perennial bushy desert plant, cushion shaped with small wrinkled green leaves [2]. It is used locally to treat inflammation and as an insect repellent [36]. The plant was tested for its antitumour, antimalarial and growth inhibition of wheat-rootlet activities [37]. In addition to the cytotoxic activity [38], and the relative toxicity of natural pyrethrins from *Pulicaria* species against *Mesocyclops leuckarti sensu lato* [39]. Aqueous extract was studied on some fish bacterial pathogens [38] and for its antioxidant activity [40]. Other studies include *in-vitro* antimicrobial activity [41]; the antileishmanicidal and the immunostimulatory effects of methanolic extract [42,43]. The chemical investigation led to isolation of xanthanolides, seco-sesquiterpene lactone, pseudoguaianolide epoxide and guaianolide [44-46] and two sesquiterpene lactones which showed cytotoxic activity [36].

Onopordum heteracanthum C. A. Mey is an erect branching spiny leaved herb, with white-downy leaves and spiny winged stems [2]. Reviewing the current literature, nothing could be traced about the phytochemical or biological studies on the plant.

In this study, the methanolic extracts of some compositae plants were tested for their *in-vitro* anti-microbial activity against different strains of bacteria and fungi, and *in-vitro* cytotoxic screening on different human cancer cell lines. The obtained results showed that, the most remarkable antibacterial activity was shown by the plant extracts of *Pulicaria crispa*, *Verbesina encelioides* and *Psiadia punctulata*. Other plant extracts under investigation as *Echinops spinosissimus*, *Euryops arabicus*, *Launaea mucronata*, *Osteospermum vaillantii* and *Onopordum heteracanthum* were less active. Only the extracts of *Pulicaria crispa* and *Psiadia punctulata* exhibited antifungal activity. The cytotoxicity study revealed that *Osteospermum vaillantii* and *Onopordum heteracanthum* have a promising cytotoxicity against different cancer cell lines.

II. RESULTS AND DISCUSSION

The results of the antimicrobial activity (Table 1) showed that, the studied plant extracts exhibited a significant antibacterial activity against the Gram-positive bacteria *S. aureus* 25913, except *Launaea mucronata*. The most remarkable antibacterial activity with inhibition zone higher than 15mm was shown with the extracts of

Verbesina encelioides (22 mm), *Psiadia punctulata* (19 mm) and *Pulicaria crispa* (24 mm). Other plant extracts as *Echinops spinosissimus*, *Euryops arabicus*, *Osteospermum vaillantii* and *Onopordum heteracanthum* were less active as antibacterial agents with inhibition zones between 10 and 15 mm. On the other hand, four plant extracts: *Verbesina encelioides*, *Launaea mucronata*, *Osteospermum vaillantii* and *Onopordum heteracanthum* exhibited low activity against the Gram-negative bacteria represented by *E. coli* ATCC 25922 with inhibition zones between 10 and 15 mm (Table 1) while, the extracts of *Echinops spinosissimus*, *Euryops arabicus*, *Psiadia punctulata* and *Pulicaria crispa* did not express any activity. Only the extracts of *Psiadia punctulata* and *Pulicaria crispa* exhibited antifungal activity against *Candida albicans* ATCC 10231, with inhibition zones of 12 mm and 20 mm respectively (Table 1) while other plant extracts were inactive. Our study is considered the first report about the antimicrobial and cytotoxic activities of *Launaea mucronata*, *Osteospermum vaillantii* and *Onopordum heteracanthum*. Although, *Echinops spinosissimus* was reported to have antibacterial and antifungal activities against *E. coli* ATCC 25922, *S. aureus* 25923 and *Candida albicans* [25] our experiment showed activity only against *S. aureus* 25923 (Table 1).

Table (1): The inhibition zones of plant extracts with different microorganisms

Plant extracts	<i>S. aureus</i>	<i>E. coli</i>	<i>Candida albicans</i>
1- <i>Echinops spinosissimus</i>	13 mm	-	-
2- <i>Euryops arabicus</i>	12 mm	-	-
3- <i>Verbesina encelioides</i>	22 mm	12 mm	-
4- <i>Launaea mucronata</i>	-	12 mm	-
5- <i>Osteospermum vaillantii</i>	12 mm	13 mm	-
6- <i>Psiadia punctulata</i>	19 mm	-	12 mm
7- <i>Pulicaria crispa</i>	24 mm	-	20 mm
8- <i>Onopordum heteracanthum</i>	15 mm	14 mm	-

Inhibition zones: ≥ 15 mm (Significantly active); 10-15mm (moderately active); ≤ 10 mm inactive

It was previously reported that the essential oil of *Euryops arabicus* showed antibacterial activity against *E. coli* (BNI 2) and *S. aureus* (BNI 18), and no activity with *C. albicans* (BNI 33) [29], but in our study we tested the total methanolic extract which showed a minor activity against *S. aureus* only. It was previously reported that *Verbesina encelioides* [35] showed antimicrobial activity against Gram-positive bacteria and *Candida albicans* than against Gram-negative bacteria and our obtained results are in a good agreement with those reported earlier [35]. *Psiadia punctulata* was reported to have antibacterial activity when tested on *S. aureus* ATCC 6538, with no activity against Gram-negative bacteria represented by *E. coli* ATCC 11229 [24]. Similarly, our obtained results are in a good agreement with those reported before [24] in addition; our study is the first report about the antifungal activity of *Psiadia punctulata* against *Candida albicans* ATCC 10231. Reviewing the current literature nothing could be traced about the cytotoxic activity of *Launaea mucronata*, *Osteospermum vaillantii* and *Onopordum heteracanthum*. Therefore, these three plants are herein subjected to such kind of study. The Chemosensitivity IC_{50} results (Table 2) showed that *Launaea mucronata* extract among the selected concentrations was not cytotoxically active against all the tested cancer cell lines with IC_{50} values >200 μ g/ml. *Osteospermum vaillantii* extract did not show any activity on MCF7 and HeLa cancer cell lines with $IC_{50} >200$ μ g/ml and weak activity on A2780 with $IC_{50} 92.36$ μ g/ml. However, it is difficult to make speculations about the possible structure and the efficacy of its constituent(s) as no previous research to isolate components was performed on this plant. *Onopordum heteracanthum* extract exhibited a remarkable and potent cytotoxic effect against A2780 (ovary cancer) and MCF7 (breast cancer) cell lines and a noticeable cytotoxic effect on HeLa (cervix cancer) cell line. This is remarkable due to the fact that different cytotoxicity levels were detected between the cells (A2780 and MCF7 were almost 4 fold more sensitive), which indicate a possible selectivity of this extract (or some of its constituents) toward certain types of cancers.

Table (2): The IC_{50} of the selected plants with different cancer cell lines

Drug and plant extract	MCF7	HeLa	A2780
5-FU	165 \pm 57.2 ng/ml	202.9 \pm 87.1 ng/ml	112.5 \pm 46.8 ng/ml
<i>Launaea mucronata</i>	>200 μ g/ml	>200 μ g/ml	>200 μ g/ml
<i>Osteospermum vaillantii</i>	>200 μ g/ml	>200 μ g/ml	92.36 \pm 5.24 μ g/ml
<i>Onopordum heteracanthum</i>	10.79 \pm 1.81 μ g/ml	41.25 \pm 7.56 μ g/ml	10.71 \pm 1.32 μ g/ml

5-FU: 5-fluorouracil; MCF7: breast cancer cell line; HeLa: cervix cancer cell line; A2780: ovarian cancer cell line

From the obtained cytotoxicity results, we can conclude that *Osteospermum vaillantii* and *Onopordum heteracanthum* have a promising anticancer activity that somehow show some selectivity toward certain cancer cell types.

In continuation of our research work on these plants, an intensive studies will be achieved including fractionation, isolation and structure elucidation of the active constituents to characterize those responsible for the cytotoxic and/or antimicrobial activity.

III. EXPERIMENTAL

3.1. Plant materials

Eight plants (*Psiadia punctulata*, *Osteospermum vaillantii*, *Echinops spinosissimus*, *Euryops arabicus*, *Launaea mucronata*, *Verbesina encelioides*, *Pulicaria crispa* and *Onopordum heteracanthum*) belong to family Compositae (Asteraceae), were collected from different localities around Taif area, KSA between Dec. 2012 and Jan 2013. The plants were kindly identified by Prof. Dr. Yassin Al-Soudany, Dept. of Botany, College of Science, Taif Univ., KSA. A voucher specimen of each plant was kept in the Dept. of Pharmacognosy, College of Pharmacy, Taif Univ., KSA.

3.2. Antimicrobial materials

3.2.1. Microorganisms, media and chemicals:

The microorganisms *S. aureus* 25923, and *E. coli* ATCC 25922 and *Candida albicans* ATCC 10231 were provided from Department of Microbiology, College of Pharmacy, Taif University, Taif, KSA. Nutrient agar, mannitol salt agar, MacConkey's agar, sabaroud dextrose agar and Muller- Hinton agar (MHA) were purchased from Difco Laboratories, U.S.A., barium chloride, sulfuric acid were purchased from Sigma Aldrich.

3.3. Cytotoxic Materials

All cancer cell lines were obtained as a kind gift from Dr. Ahmad Aljada, Department of Basic Medical Sciences, king Saud bin Abdulaziz University for Health Sciences, KSA

3.4. Preparation of the crude plant extracts

Fifty grams of each of the air-dried powdered plant materials was separately extracted with methanol till exhaustion using soxhlet apparatus. The total methanolic extracts were separately concentrated under vacuum using rotary evaporator till dryness to afford dry extracts as follow: *Psiadia punctulata* (17.5 g), *Osteospermum vaillantii* (8.9 g), *Echinops spinosissimus* (5.8 g), *Euryops arabicus* (15.2 g), *Launaea mucronata* (6.6 g), *Verbesina encelioides* (8.0 g), *Pulicaria crispa* (8.7 g), *Onopordum heteracanthum* (6.5 g).

3.5. Assay of the antimicrobial activity by disc diffusion method:

Antibacterial assay was performed by disc diffusion method (modified Kirby -Bauer method) according to [47] and the extracts that showed inhibition zones ≥ 15 mm were considered significantly active, while those giving ≤ 10 mm diameter zones were considered inactive.

3.6. In-vitro cytotoxic screening of the plant extracts against some human cancer celllines:

3.6.1. Chemosensitivity

All plant extracts were dissolved in *DMSO* to obtain stock solutions at maximum soluble concentration (200 mg/ml), stored at room temperature prior to use. Breast (MCF7) and ovarian (A2780) cancer cell lines were routinely maintained as mono-layered cultures in RPMI 1640 medium, cervical cancer cells (HeLa) were routinely maintained as mono-layered cultures in DMEM-high glucose medium. All media were supplemented with 10% fetal bovine serum, 2 mM L-glutamine and 1 mM sodium pyruvate. The arrest of cell growth was determined by the methylene blue-based cytotoxicity assay as previously reported [48]. Briefly, cancer cells at 3000 cells/well were seeded into a 96-well plate and allowed to adhere overnight. Cells were then exposed to a range of drug concentration for 96 h. After the incubation period, the medium was discarded and methylene blue solution (150 μ l, 0.5% w/v in ethanol:water; 50:50, v/v) were added to each well and incubated at dark for 1 h at room temperature. Unbound methylene blue was washed off with distilled water and bound stain was solubilized by the addition of 200 μ l of 1% acetic acid solution (glacial acetic acid:ethanol:water; 1:50:49, v/v/v). The plates were agitated on a plate shaker at 200 rpm/min for 1 h at room temperature. The absorbance in each well was read at 630 nm in multiwell plate spectrophotometer (BioTek, USA). The average absorbance in the control wells was taken as 100% survival, and the IC_{50} values were defined as the drug concentrations that inhibited the cell growth by 50% after 96 h drug exposure.

IV. ACKNOWLEDGEMENT

The authors are grateful to the staff members of the Department of Microbiology, College of Pharmacy, Taif University, Taif, KSA for providing the facilities for antimicrobial study.

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