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Contributed Paper

Selective Biological Activities and Phytochemical Profiling of Two Wild Plant Species, *Teucrium Polium* and *Capsicum Annum* from Sheringal, Pakistan

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Abstract

In this study, various extracts (hexane, acetone, dichloromethane, ethyl acetate and ethanol) of two medicinally important wild plant species, *Teucrium polium* and *Capsicum annum* were investigated for their antioxidant and antimicrobial potential. The ethanol extracts of *T. polium* and *C. annum* showed maximum inhibition at IC_{50} values of 135±1.09 and 70±0.08 respectively in 1,1-diphenyl-2-picryl-hydrazyl (DPPH) scavenging assay. Interestingly, in (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay, the ethyl acetate extract of *T. polium* showed potent antioxidant effect with IC_{50} value of 125±0.32, while the ethanol extract of *C. annum* exhibited IC_{50} value of 140±1.15. Ethanol extract of *T. polium* was active in inhibition of *Escherichia coli* up to 73 % as well as effective against fungi, *Aspergilus flavus* with 70 % inhibition in antifungal assay. In case of *C. annum*, ethyl acetate extract was potent in inhibiting *Bacillus subtilis* (73 %) while ethanol extract effectively inhibited the linear growth of *A. flavus* and *T. longifusus* by 77 and 70 % respectively. These interesting results may be attributed to the presence of flavonoids and terpenoids in *T.polium,* while saponins and phlobatannins were the main constituents in *C.annum.*

Key words: *Teucrium polium, Capsicum annum,* DPPH and ABTS free radical scavenging assay, antimicrobial activities, phytochemical screening

1. Introduction

Teucrium is a genus of wild growing perennial herbaceous flowering plants (340 species) and belongs to family Lamiaceae, found throughout the world and native to Mediterranean region [1]. Various parts of the plant *Teucrium polium* have been used as anti-hypolipidemic, anti-inflammatory, anti-rheumatoid, anti-pyretic, anti-spasmodic [2-3]. Recent studies show that *T. polium* can prevent cell invasion and motility of human prostate cancer cells through different molecular pathways [4].

Capsicum annum is the most important and widely found species with sweet and peppery fruit. *C. annum* belongs to the family, Solanaceae, commonly used all over the world as peppers. *Capsicum* species have been known to contain high amount of vitamins C and carotene (provitamin A). *C. annum* is best known for its use in traditional and local medicine such as anti-inflammatory and analgesic, due to the presence of capsaicinoids. Other medicinal uses of this plant include carminative agent and for the ailment of rheumatism, lumbago, and neuralgia [5-6]. Pharmacologically, cis-capsaicin (civamide) obtained from *Capsicum* species is reported to be active against herpes simplex treatment in guinea pigs and has also been clinically tested for relieving migraine headache thus showing good effects on sensory neurons [7].

This study evaluates the antioxidant and antimicrobial potential of various extracts obtained from *T. polium* and *C. annum* wild species collected from Sheringal valley, Pakistan. Phytochemical profiling of all the extracts was also carried out alongside.

2.1 MATERIALS AND METHODS

2.1 Plant Material

The whole plant of *T. polium* and ripped fruits of *C. annum* were collected from Sheringal valley, Dir (U), Pakistan in June 2015 and were identified by Dr. Ali Hazrat, Lecturer, Department of Botany, Shaheed BB University, Wari campus.

2.2 Preparation of Plant Extracts

The plant materials were shade dried, chopped and milled to powder (450 g). 90 g of each material were separately soaked with *n*-hexane, dichloromethane (DCM), acetone, ethyl acetate and ethanol (700 mL each) in cold maceration method while keeping for 7 days at room temperature. The infusions were filtered through Whattman filter paper (No-1) and concentrated to solid mass on rotary evaporator to obtain *n*-hexane, acetone, DCM, ethyl acetate and ethanol soluble extracts of *T. polium* and *C. annum* respectively. All the solvents and chemicals used were of analytical grade.

2.3 *In vitro* Antioxidant Assay

2.3.1 DPPH (1, 1-diphenyl-2-picryl-hydrazyl) free radical scavenging activity

DPPH free radical scavenging assay was performed according to the method described in literature [8-9]. Briefly, 0.1 mM solution of DPPH was prepared in methanol. In a glass vial, 1 mL of this solution was mixed with 3 mL of each extract sample and different concentrations i.e. 50, 100, 150 and 200 μg/mL were prepared. The reaction mixtures were mixed well and kept in dark for one hour at a controlled temperature (37°C). Spectrophotometric absorbance of reaction mixture was measured at 517 nm. 3 % methanol was used as a blank while mixture of 100 μL 3% methanol sample in 900 μL of DPPH were taken as negative control. Ascorbic acid was used as positive control.

2.3.2 ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) method

ABTS assays were performed by using the antioxidant Assay Kit (Cat. No CS0790, Sigma- Aldrich) in ultrapure water while the antioxidant, trolox was used as reference standard [10]. ABTS substrate and working solution were prepared by adding 25 μL of 3% hydrogen peroxide solution (Cat. No 323381, Sigma- Aldrich) to 10 mL of ABTS substrate solution for a standard curve. The solution was used within 20–30 minutes. 10 μL of trolox and 20 μL of myoglobin working solution were added to the wells to obtain trolox standard curve followed by the addition of 10 μL test samples and 20 μL of myoglobin working solution to the wells for the test samples. In step 2, 150 μL of ABTS substrate working

solution was added to each well and incubated for 5 minutes at room temperature, proceeded by the addition of 100 µL of stop solution (Cat. no S3446, Sigma-Aldrich) to each well. The endpoint was monitored as absorbance at 405 nm, using a plate reader.

2.4 Antimicrobial Assays

2.4.1 Antibacterial assay

Antibacterial activity was carried out by slight modification of agar disc diffusion method [11]. Bacterial strains used in this assay were *Escherichia coli* (ATCC 15224), *Bacillus subtilis* (ATCC 6663), *Staphylococcus aureus* (ATCC 29213) and *Shigella flexneri* (ATCC 14028). Stock solutions of the test samples having a concentration of 1 mg/mL were prepared in sterile DMSO. 100 µL and 200 µL of each dilution were added to the respective wells. The control contained 100 µL and 200 µL DMSO. Clarithromycin was used as standard drug and positive control. The plates were left at room temperature to allow diffusion and then incubated at 37 °C for 24 hr. The diameter of the zones of inhibition was measured in mm. Minimum inhibitory concentration (MIC) of antibiotics was carried out by standard micro broth dilution method [12].

2.4.2 Fungicidal assay

The antifungal activity of the plant extracts was carried out by agar dilution method [13]. Fungal strains used in this study were *Aspergillus flavus* (ATTC 32611), *Aspergillus niger,* clinically isolated, obtained from Microbiology Lab, Agha Khan University Hospital, Karachi), *Candida albicans* (ATTC 2091) and *Trichophyton longifusus* (ATTC 22397). The samples (24 mg/mL) were dissolved in sterile DMSO. Inhibition of fungal growth was observed visually after 7 days of incubation at 37 °C, placed an open pan of water in an incubator to control humidity (40–50%). Media growth was determined by measuring linear growth (mm) and growth inhibition was calculated with reference to the negative control.

2.5 Phytochemical Screening

Chemical tests were carried out on all the fractions of both plants using standard procedures to identify the phyto-constituents as described by Masood *et al.* (2013) [14].

3. RESULTS

3.1 DPPH Free Radical Scavenging Activity

Table-1 illustrates DPPH free radical scavenging activity results reported for *T. polium* and *C. annum*. The results reveal that ethanol extracts of *T. polium* exhibited promising antioxidant potential with IC_{50} value of 135±1.09. In case of *C. annum*, ethanol and acetone extracts showed excellent inhibition at IC_{50} = 70±0.08 and 80±0.01 µg/mL respectively. The remaining extracts encompassed weak antioxidant effects against DPPH free radical as compared to ascorbic acid (standard).

3.2 ABTS % Scavenging Activity

In concentration dependent ABTS % scavenging assay (Table-2), ethanol extract of *T. polium* showed maximum potency, 76 % at concentration of 200 µg/mL and promising IC_{50} value of 145±0.81 µg/mL, while the ethanol extract of *C. annum* was potent with the IC_{50} value of 140±1.15 µg/mL. The ethyl acetate extract of *T. polium* demonstrated potent inhibition of 70 % at a concentration of 200 µg/mL with IC_{50} value of 125 µg/mL while the remaining extracts showed mild to nil inhibitory effect. Trolox was used as reference.

3.3 Antimicrobial Activities

All the extracts of *T. polium* and *C. annum* were screened for their antibacterial activities. Representative of gram positive and gram negative bacteria including *E. coli, B. subtilis, S. aureus* and *S. flexneri* were used as tested bacteria. The results (Table-3) indicated that

Table 1. DPPH (%) scavenging antioxidant activities of *T. polium* and *C. annum* extracts.

DPPH % scavenging of *T. polium* extracts						
Concentrations (µg/mL)	**Reference Standard (Ascorbic acid)**	**Test samples**				
		Hexane	**Acetone**	**DCM**	**Ethyl acetate**	**Ethanol**
50	**60**	12 ± 0.01	20 ± 0.07	21 ± 0.02	24 ± 0.05	33 ± 0.12
100	**75**	24 ± 0.15	28 ± 0.08	34 ± 0.01	38 ± 0.10	48 ± 0.14
150	**100**	30 ± 0.50	39 ± 0.05	42 ± 0.10	48 ± 0.11	58 ± 0.01
200	**100**	39 ± 0.08	46 ± 0.02	51 ± 0.06	60 ± 0.01	69 ± 0.13
IC_{50} (µg/mL)	**40±0.01**	------	------	**170±1.05**	**185±1.5**	**135±1.09**
DPPH % scavenging of *C. annum* extracts						
		Hexane	**Acetone**	**DCM**	**Ethyl acetate**	**Ethanol**
50	**60**	15 ± 0.11	19 ± 0.11	30 ± 0.11	25 ± 0.91	35 ± 0.18
100	**75**	26 ± 0.19	25 ± 0.15	33 ± 0.16	30 ± 0.21	50 ± 0.02
150	**100**	33 ± 0.5	55 ± 0.02	41 ± 0.07	50 ± 0.11	65 ± 0.09
200	**100**	40 ± 0.3	70 ± 0.4	48 ± 0.13	81 ± 0.20	78 ± 0.03
IC_{50} (µg/mL)	**40±0.01**	------	**80**±0.01	------	**82**±0.02	**70**±0.08

Note: Values shown are mean ± SEM, No. of experiments = 3.

Table 2. ABTS (%) scavenging of *T. polium* and *C. annum* extracts.

ABTS % scavenging of T. polium extracts						
Concentrations (µg/mL)	**Reference standard (Trolox)**	**Test samples**				
		Hexane	**Acetone**	**DCM**	**Ethyl acetate**	**Ethanol**
50	------	11 ± 0.02	12 ± 0.18	18 ± 0.03	20 ± 0.07	37 ± 0.06
100	------	17 ± 0.05	25 ± 0.25	27 ± 0.08	39 ± 0.12	43 ± 0.09
150	------	20 ± 0.04	45 ± 0.70	35 ± 0.30	56 ± 0.18	61 ± 0.02
200	------	25 ± 0.01	58 ± 0.4	47 ± 0.08	70 ± 0.05	76 ± 0.19
IC_{50} (µg/mL)	1.70±0.48	------	160±0.60	------	125±0.32	145±0.81
ABTS % scavenging of C. annum extracts						
		Hexane	**Acetone**	**DCM**	**Ethyl acetate**	**Ethanol**
50	------	18± 0.07	20± 0.05	14±0.01	22± 0.3	30± 0.02
100	------	20± 0.05	32± 0.04	25± 0.06	20± 0.09	35± 0.01
150	------	33± 0.03	40± 0.12	43± 0.15	26± 0.2	56± 0.25
200	------	44± 0.11	52± 0.23	58± 0.19	59± 0.4	69± 0.31
IC_{50} (µg/mL)	1.0±0.53	------	165±0.11	172±0.15	175±1.20	140±1.15

Note: Values shown are mean ± SEM, No. of experiments = 3

ethanol extract of *T. polium* showed strongest antibacterial activity i.e. 73 % against *B. subtilis,* 57 % against *E. coli* and 45 % against *S. flexneri* respectively. The ethyl acetate extract remained active against *B. subtilis* with 56 % inhibition while the other extracts of this plant showed moderate to nil effects. In case of *C. annum*, ethanol extract pronounced 73 % inhibition against *S. subtilis* and 57 % against *E. coli* while was inactive against the rest of bacteria. These results were compared with standard drug clarithromycin.

The fungicidal activities of *T. polium* and *C. annum* extracts against four pathogenic fungi i.e. *A. flavus*, *A. niger*, *C. albicans* and *T. longifusus* have been summarized in Table 3. The results reveal that ethanol and ethyl acetate extracts of *T. polium* were potent in inhibition the growth of *A. flavus* up to 70 and 65 % respectively. The ethyl acetate extract also inhibited the linear growth of *C. albicans* by 60 % and *A. niger* by 50 %. The other extracts remained inactive. The antifungal effect was better demonstrated by the extracts of *C. annum*. According to the results, ethanol extract inhibited the growth of *A. flavus* and *T. longifusus* 77 and 70 % respectively while ethyl acetate extract of *C. annum* remained active against both *A. flavus* and *A. niger* up to 66 and 68 % respectively.

3.4 Phytochemical Screening

The phytochemical tests for the presence of various secondary metabolites including glycosides, reducing sugars, saponins, flavonoids, phlobatannins and terpenoids were performed for all the extracts of *T. polium* and *C. annum*. In *T. polium,* all extracts reveal the presence of terpenoids, flavonoids and anthraquinones In contrast; extracts of *C. annum* gave positive tests for the presence of saponins and phlobatannins along with minor amount of flavonoids and reducing sugars (Table-4).

Table 3. Antimicrobial activities of *T. polium* and *C. annum* extracts.

% Inhibition												
Bacteria	**Z.I Standard (Clarithromycin)**	**MIC mg/mL**	**Ethanol**		**Ethyl acetate**		**DCM**		**Acetone**		**Hexane**	
			TP	**CA**	**TP**	**CA**	**TP**	**CA**	**TP**	**CA**	**TP**	**CA**
E. coli	35	0.235	57	-----	42	57	37	42	31	31	30	20
B. subtilis	23	0.162	73	-----	56	73	----	43	31	----	----	----
S. aureus	29	0.225	37	24	24	37	----	24	----	----	10	31
S. flexneri	20	0.155	45	25	25	45	25	----	30	25	35	30
LG (mm) in %												
Fungi	**Standard Drug (Terbinafine) LG (mm)**		**TP**	**CA**	**TP**	**CA**	**TP**	**CA**	**TP**	**CA**	**TP**	**CA**
A. flavus	100		70	77	65	66	0	19	25	0	0	0
A. niger	105		50	45	10	68	58	0	20	0	0	0
C. albicans	85		45	40	60	55	40	37	0	30	18	15
T. longifusus	70		40	70	0	0	27	0	15	25	5	20

TP= *T. pollium*; CA= *C. annum*; Z.I= Zone of inhibition (mm), LG= Linear growth (in mm)

The plates were inoculated at concentration of mg/mL of DMSO.

Table 4: Results of phytochemical tests of *T. polium* and *C. annum* various extracts.

S.NO	Class	Hexane		Acetone		DCM		Ethyl acetate		Ethanol	
		TP	CA	TP	CA	TP	CA	TP	CA	TP	CA
1	Glycosides	-	-	-	++	-	+	++	-	+	+
2	Reducing sugars	-	-	-	-	-	-	-	++	-	++
3	Saponins	-	-	-	++	-	++	-	++	+	++
4	Flavonoids	+	+	+	+	+	+	+	+	++	-
5	Phlobatannins	-	+	-	++	-	++	-	-	+	-
6	Terpeniods	++	-	++	-	++	-	++	-	++	-
7	Anthraquinones	+	-	+	-	-	-	+	++	+	-

Key: (++) = Strongly present, (+) = Present, (-) = Not detected

TP= *T. pollium*; CA= *C. annum*

4. Discussion

The medicinal plants found in Pakistan have been imparting significant role in treatment of various diseases at local level which include nephrotoxicity pulmonary oxidative damages, cardio toxicity, antioxidant, adrenal toxicity [15-17]. According to recent studies, about 30% of antioxidant supplements throughout the world are prepared from medicinal plants [18-19]. In continuation of these, the antioxidant studies of various extracts of wild plants of *T. polium* and *C. annum* fruits were carried out to identify their possible role as antioxidants. These medicinal plants have been in use for long time and still in used as primary health care. Our study reveals that the ethanol extract of *T. polium* and *C. annum* bears potent antioxidant effect and can be used as safe natural antioxidants in herbal products.

The activities are strongly supported by the presence of flavonoids and terpenoids in the extracts of *T. polium* and presence of saponins in *C. annum*. The results showed close similarities to the earlier study reported by Hagerman et al. (1998) [20].

In antibacterial bioassay of *T.polium*, maximum inhibition was observed against Gram- positive bacterium, *B. subtilis*, indicating the potential use of this plant in treatment of *B. subtilis* infections such as tissue necrosis, pneumonia and other musculoskeletal disorders (MSDs) [21]. Our study suggests a possible therapeutic use of both the plants extracts in treatment of various fungal diseases caused by *Aspergillus* species such as ear infections, temporary hearing loss which can damage the ear canal and tympanic membrane. Other infections include otomycosis [22], aspergillosis and endobronchial lung cancer [23]. The ethanol extract of *C. annum* shows activity against *T. longifusus* which is the causative agent of dermatophytosis and infects the hair, skin, and nails [24]. According to these results, different extracts of these medicinal plants possess compounds with antimicrobial properties which can be used as antimicrobial agents and as new drugs for therapy of infectious diseases in human. Parallel antibacterial activity of numerous other plant extracts has been documented earlier. The current work confirms that different extracts of *T. polium* and *C. annum* contain antibacterial terpenoids and saponins. These results are similar to the finding of previously reported study [25]. Overall both plant extracts showed the presence of terpenoids, saponins, phlobatannins and flavonoids which indicates the possibility of its antioxidant and antimicrobial effects.

Conclusions

Different extracts obtained from wild medicinal plants, *T. polium* and *C. annum* were assayed for their antioxidant potential as well as antimicrobial activities. The ethanol extracts showed maximum antioxidant activity in both DPPH and ABTS free radical scavenging assays probably due to the presence of terpenoids, flavonoids and saponins. In antibacterial bioassay, ethanol and ethyl acetate extracts exhibited maximum inhibition against *B. subtilis* thus suggests their probable uses in the treatment of diseases like pneumonia and other diseases caused by these species. Both ethanol and ethyl acetate extracts were much active in the inhibition of *Aspergillus* species in antifungal assay. When screened for their phytochemicals, majority of the extracts showed the presence of terpenoids, flavonoids as well as saponins. The results confirm a possible use of *T.polium* and *C. annum* as antioxidant, antibacterial and antifungal agents. Further studies are recommended for the purification of phytochemicals responsible for these activities.

Conflict of interest

The author's declared no conflict of interest.

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