Hindawi
Journal of Chemistry
Volume 2022, Article ID 6486717, 12 pages
https://doi.org/10.1155/2022/6486717

Research Article

Antioxidant, Antimicrobial, and Protein Kinase Inhibition Profiling of C. ambrosioides Seed Extracts along with RP-HPLC

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Received 10 January 2022; Revised 14 March 2022; Accepted 22 March 2022; Published 25 April 2022

Academic Editor: Jayant Kumar Patra

The validation of underexplored traditional plant remedies represents a reservoir of novel leads for drug discovery. In line with this, in vitro total phenolics and flavonoids content, multimode antioxidants, antimicrobial, cytotoxicity, and protein kinase inhibition assays were conducted on C. ambrosioides seed extracts in addition to RP-HPLC. Methanol extract exhibited highest total phenolics ($64.6 \pm 0.6\,\mu$g gallic acid equivalent/mg) and flavonoids ($50.9 \pm 0.5\,\mu$g quercetin equivalent/mg) content. RP-HPLC quantified rutin ($1.98\,\mu$g/mg) in methanol extract whereas quercetin ($0.322\,\mu$g/mg) and kaempferol ($2.86\,\mu$g/mg) in methanol-distilled water extract. Methanol extract exhibited highest ascorbic acid equivalent (AAE) free radical (DPPH) scavenging (IC_{50} of $110.7 \pm 5\,\mu$g/ml), total antioxidant capacity ($110.6 \pm 2.2\,\mu$g AAE/mg), and total reducing power ($94.30 \pm 0.46\,\mu$g AAE/mg). Highest antibacterial activity against K. pneumonia (14 ± 1.61 mm ZOI) and antifungal activity against F. solani (17 ± 1.38 mm ZOI) were shown by n-hexane and chloroform extracts, respectively. Ethyl acetate extract exhibited highest brine shrimps cytotoxicity (LC_{50} of $125\,\mu$g/ml). A noteworthy protein kinase inhibitory potential was shown by ethanol extract with a 20 ± 1.27 mm bald zone. Therapeutic potential of medicinal plants can be completely explored by using multiple solvent system. This study makes C. ambrosioides, a resourceful prospect for the bioactivity-guided isolation of lead compounds.

1. Introduction

Plants have formed the basis for a sophisticated traditional system of medicines that paved the way to provide potential remedies for various ailments [1]. Wide spectrum biological properties possessed by plant secondary metabolites have made them an important source of bioactive leads. The chemical diversity and versatility of plant-based remedies emphasize the need of critical screening via modern approaches based on ethnopharmacological fingerprints. Advancements in the science of drug discovery have led to effective scrutiny of plants having potential medicinal benefits. It reinvigorated pharmaceutical firms and practitioners towards plant-oriented research and instilled ardor into quest of new drug moieties [2].

Evaluation of numerous plant species and herbs showed that if antioxidant potential is present in plants then they can be used for the treatment of cardiovascular diseases, hyperglycemia, Alzheimer, and even cancer. Teas, vegetables, and fruits prevent serious health complaints like cardiovascular diseases and cancer owing to the presence of antioxidants and numerous other chemical entities. Vitamins, carotenoids, anthocyanins, flavonoids, and various other polyphenolic components are the keystones of antioxidant, antimicrobial, and anticancer potential of plant products [3].

Genus Chenopodium belongs to the family chenopodiaceae. It has 102 genera and 1700 species [4]. Chenopodium ambrosioides (wormseed) is an annual or perennial erect branched aromatic herb indigenous to subtropical and temperate regions including Central America, South America, and Brazil [5]. It is naturally growing in Margalla hills of Pakistan [6] and is commonly known as Gandi Buti [7]. For centuries, different preparations of C. ambrosioides have been utilized by native people due to its dietary and medicinal importance. It is rich in terpenoids and flavonoids components that exhibit profound pharmacological activities such as anthelmintic, antioxidant, antitumoral, antileshmanial, antiinflammatory, wound healing, and cancer chemoprevention [8]. In Brazil, this folk medicine is used to treat respiratory problems, tuberculosis, and rheumatism [5] and has also proved effective in the treatment of uterine hemorrhage [9]. The essential oil (chenopodium oil) obtained through hydrodistillation of the plant is a mixture of ascaridole, isoascaridole, p-cymene, limonene, and alpha terpinene with known medicinal values [10–12]. Despite already documented phenomenol biological attributes of C. ambrosioides, its restorative potential is further explored in the present study. Fourteen solvent systems having a wide polarity distribution were used on the seeds to make extracts. To the best of our knowledge, this is the first report on RP HPLC, antibacterial, antifungal, cytotoxic, and protein kinase inhibitory potential of seed extracts.

2. Methodology

2.1. Protocols Adopted Are Well Developed and Cited Properly

2.1.1. Collection and Identification. Plant material was collected from the vicinity of the Quaid-i-Azam University Islamabad and identified as C. ambroisides by Professor Dr. Rizwana Aleem Qureshi. A voucher specimen (PHM-494) was deposited at the herbarium of medicinal plants, Quaid-i-Azam University Islamabad, Pakistan.

2.1.2. Preparation of Crude Extracts. Deteriorated seeds were removed, rinsed with tap water, shade dried for four weeks, and then pulverized to coarse powder. Extraction was carried out in 14 different solvents of varying polarity either alone or in 1 : 1 proportion which includes n-hexane (NH), chloroform (CHL), ethyl acetate (EA), acetone (Act), methanol (Me), ethanol (Eth), distilled water (Dw), ethyl acetate-n-hexane(EA-NH), ethanol-ethyl acetate (Eth-EA), chloroform-methanol (CHL-Me), ethyl acetate-methanol (EA-Me), acetone-methanol (Act-Me), acetone-distilled water (Act-Dw), and methanol-distilled water (Me-Dw). The weighted amount of plant powder (60 g) was macerated in different solvent systems separately, followed by intermittent shaking and ultrasonication. On the third day, it was filtered by muslin cloth and fine filtered using Whatman no. 1 filter paper. The filtrates were evaporated to dryness in a rotary evaporator at 45°C under reduced pressure. The process was repeated twice by using the same individual marcs. The dried crude extracts thus obtained were combined and stored in preweighed vials at -80°C till further analysis.

Extract recovery was calculated as

%Extract recovery = $(A/B) \times 100$.

A = weight of crude extract.

B = weight of the respective powdered plant material.

2.2. Phytochemical Analysis

2.2.1. Total Phenolics Content Determination (TPC). The phenolic content in the test sample was determined by the use of a 10 percent phenol-Ciocalteu (FC) agent, as described [13]. Gallic acid and DMSO have been used as positive and negative standards. The extract solution (20 μl; 4 mg/ml DMSO) and FC solution (9 : l) were mixed in 96-well plates. After 5 minutes, add 90 μl sodium carbonate (6% w/v), incubate for 30 minutes at 37°C (Germany), and record the absorption at 630 nm with a microplate reader. The calibration curve is calculated using gallic acid (2.5, 5, 10, and 20 μg/ml). The process was repeated twice. The resulting phenolic content is expressed as equivalent to μg gallic acid per mg extract (μg GAE/mg).

2.2.2. Total Flavonoids Content Determination (TFC). In 96 well plates, mix 20 μl sample solution (4 mg/ml DMSO), 10 μl of 1 M potassium acetate (98.15 g/L), 10 μl of aluminium chloride (10% w/v), and 160 μl of distilled water was incubated at room temperature for 30 min. Finally, the microplate reader is set, and the absorption is measured at 415 nm. The calibration curve is drawn with quercetin at a final concentration of 2.5, 5, 10, 20, and 40 μg/ml. And the resulting flavonoids were expressed after triple analysis in the form of an equivalent of μg of quercetin per mg extract (μg QE/mg) [14].

2.3. RP-HPLC. RP-HPLC with analytical columns has been used to quantify polyphenols using the methodology described [13, 15]. Reference compounds, i.e., catechins, quercetin, gallic acid, caffeic acid, myricetin, rutin, apigenin, and kaempferol; the final concentration was prepared with samples diluted by methanol to 50 μg/ml. Two mobile phases were used, the mobile phase A contains 5 : 10 : 85 : 1 acetonitrile-methanol-water- acetic acid, while the mobile phase B contains 40 : 60 : 1 acetonitrile-methanol-acetic acid. The flow rate was kept at 1 ml/min. Each sample contains 20 μl aliquots (10 mg/ml methanol) in the column, which is allowed to be reconditioned for 10 minutes before the next analysis. Mobile phase A is isocratic, and B gradient volume is 0–50% in 0-20 minutes, 50–100% in 20-25 minutes, and 100% in 25-30 minutes. The samples were shown absorbance at different wavelengths, e.g., rutin was determined at 257 nm, gallic acid and catechin at 279 nm, apigenin and caffeic acid at 325 nm, and kaempferol, quercetin, and myricetin at 368 nm.

3. Biological Evaluation

3.1. Free Radical Scavenging (DPPH) Assay. The sample solution (20 μl, 4 mg/ml DMSO) and 180 μl of the DPPH agent (9.2 mg/100 ml ethanol) were added to the 96 wells,

and then the incubation at 37°C for 1 hour in the dark cabin. The absorption was measured using a microplate reader at 517 nm. Ascorbic acid is the reference standard. The experiments were repeated three times. The percentage of sample radical bleaching potential is calculated by this formula:

$\%\text{scavenging activity} = (1 - \text{Ab}_s/\text{Ab}_c) * 100.$

Ab_s = absorbance of DPPH solution with sample.

Ab_c = absorbance of negative control (containing the reagent solution without sample).

A sample with a scavenging of >50% at 400 μg/ml was tested in lower concentrations using three-fold serial dilution methodology to find the IC_{50} value. The corresponding IC_{50} values are calculated using the table curve software [16, 17].

3.2. Total Antioxidant Capacity Determination. A 100 μl sample solution (DMSO 4 mg/ml) was mixed with a 1 ml mixture (0.6 m sulfuric acid, 4 mM ammonium molybdate, and 28 mM sodium phosphate), incubated at 90° C for 95 min, and cooled at room temperature, and absorbance was taken at 645 nm using a spectrophotometer. Ascorbic acid is used as a positive control agent, and DMSO is used as a negative control agent. The antioxidant potential of each solvent extract is calculated after three-step analysis, and the result is expressed as microgram (μg AAE/mg) equivalent to the mg equivalent [13].

3.3. Total Reducing Power. A mixture containing 200 μl of each sample (4 mg/ml DMSO), 500 μl of 0.2 M phosphate buffer (pH 6.6), and 1% potassium ferricyanide [$K_3Fe(CN)_6$] was incubated for 20 min at 50°C. Afterward, 500 μl of 10% TCA was added, centrifuged at 3000 rpm for 10 min, supernatant fluid (500 μl) was transferred to Eppendorf tube, mixed with ferric chloride (500 μl), and distilled water (100 μl). Absorbance was recorded at 700 nm. DMSO and ascorbic acid (4 mg/ml) were used as negative and positive controls. The results were represented as μg ascorbic acid equivalent per mg of extract (μg AAE/mg) after triplicate analysis [16].

3.4. Antimicrobial Assays. The first activity of the test sample against bacterial and fungal strains has been investigated using the diffusion method of agar discs [14]. About 100 μl of each test strain is distributed on a presteriled agar plates. The filter paper disc (6 mm diameter) was infused with a test extract of 5 μl (100 μg) each. Afterward, discs were placed on previously seeded agar plates. The discs impregnated with (4 mg/ml of DMSO) and clotrimazole (4 mg/ml of DMSO) served as positive controls, while the DMSO-injected discs served as negative controls. After 24 hours at 37°C (antimicrobial) and 24 hours at 25°C (antimicrobial) incubation, the diameter and control of the growth inhibition zone around the sample were measured to the closest mm via vernier caliper and recorded after triple analysis of measuring.

3.5. Brine Shrimp Toxicity Assay. Hatched nauplii (Artemia salina) in sea water (34 g/L sea salt + 6 mg/L yeast) were collected. Different subdilutions of the test sample were tested to determine the lethal concentration at 1000, 500, 250, and 125 μg/ml. A precise count of 20 nauplii were transferred to each well-containing seawater. The corresponding microliters of each concentration were added to each well, and the final volume of each well was made up to 300 μl with seawater. DMSO concentration did not exceed 1%. Doxorubicin and DMSO are positive and negative controls. After 24 hours of incubation at 30°C, the plates were examined with a reverse microscope, and the dead nauplii that were settled at the bottom were counted in each well. LC_{50} was calculated accordingly for the extracts with ≥50% mortality at highest concentration using table curve software 2D version 4.

3.6. Protein Kinase Inhibition Assay. Protein kinase inhibition potential of sample extracts was evaluated according to the procedure reported previously [16, 18]. The 24-hour regenerated Streptomyces culture (100 μl) in Trypton soy broth spread to small-scale ISP4 plates on the lawn. A 6 mm diameter sterile filter paper disc was loaded with a test sample of 5 μl (100 μg) and placed on a newly planted plate. The surfactant-wetted discs function as positive controls, while the DMSO-infused discs function as negative controls. These plates were incubated for 72 hours and allowed hyphae to develop. After incubation, the growth inhibition zone is measured and recorded around each disc with a vernier caliper. The development of a bald area around the disc suggests that phosphorylation inhibition is possible by the samples. The bald area shows that hyphae formation is inhibited, while the clear area shows that Streptomyces killing thus exhibiting the cytotoxicity of the sample.

3.7. Statistical Analysis. The results of cytotoxic, antimicrobial, enzyme, and phytochemical studies were expressed as a mean ± SD in three-fold analysis. Further statistical analysis is carried out using a one-way variance analysis (ANOVA) using Statistix 8.1.

4. Results and Discussion

4.1. Percentage Yield. In total, 14 different extracts of the seeds of C. ambrosioides have been prepared with different solvents. Maximum extract yield (10.01% *w*/*w*) was obtained when Me-Dw was used as the extraction solvent (Table 1). Minimum yield was given by NH extracts, i.e., 2.17%. The solvent system, the plant material, the extraction technique, and the extraction time strongly influence the recovery of bioactive constituents [19]. Biological activities do not correspond with the extract yields, and low-yielding solvents might show more noticeable affects. It depends on distinct solubilities of diverse plant metabolites in different solvents [20]. This information is critical to large-scale extraction optimization after sound activity observation [2].

4.2. Total Phenolics Content Determination. The results of the phenolic content showed that Me was the most capable solvent to extract polyphenols (Figure 1; Table 2). Maximum phenolics content was quantified in the Me extract (64.6 ± 0.6 μg GAE/mg). On the contrary, the NH extraction quantified the lowest concentrations of phenolic acids, i.e., 3.2 ± 0.3 μg GAE/mg. The consumption of plant phenolics is associated with a decrease in the incidence of cancer, degeneration, and heart disease [21, 22] because they act as a singlet oxygen suppressor, metal chelator, and reduction

Table 1: The percentage of extract recovery from C. ambrosioides seeds extracted with various solvents.

Sr. no	Solvent extract code	% extract recovery
1	NH	2.17
2	CHL	5.00
3	EA	5.83
4	Act	5.17
5	Me	5.00
6	Eth	5.33
7	Dw	7.67
8	EA-NH	2.21
9	CHL-Me	8.33
10	EA-Me	6.17
11	Eth-EA	5.67
12	Act-Me	5.83
13	Act-Dw	8.50
14	Me-Dw	10.01

agent. The antioxidant potential may be caused by the presence of hydroxyls, methyls, double bonds, or ketones in the phenolic molecules [23].

Values are presented as mean ± standard error after triplicate analysis. (a)–(e) represent the significance of results. Samples that display different alphabets were significantly different at $p < 0.05$.

4.3. Total Flavonoids Content Determination. The determination of the total flavonoids content indicated that the highest quantity of quercetin-equivalent (QE) flavonoids in the Me extract of the seeds was quantified, i.e., $50.9 \pm 0.5\,\mu$ g QE/mg (Figure 1; Table 2). NH extract showed minimum flavonoids detection, i.e., $1.5 \pm 0.3\,\mu$g QE/mg. Flavonoids have antioxidant effects because they have a high antioxidant potential by removing and stabilizing free radicals involved in the oxidation process [24].

4.4. RP-HPLC. The raw extracts are quantitatively analyzed using reverse-phase HPLC technology to identify and quantify polyphenols. The sample peak is compared to the retention time of the reference compounds (caffeic acid, rutin, gallic acid, quercetin, apigenin, catechin, myricetin, and kaempferol) and the UV absorption spectrum. Polyphenols were detected only in Me and Me-Dw extracts, respectively.

The HPLC quantitative profiling shows that rutin is quantified in the extracts of Me seeds, i.e., 1.98 μg/mg extract (Figure 2(b); Table 3). Significant amounts of quercetin and kaempferol were quantified in Me-Dw (0.322 and 2.86 μg/mg, respectively) extract of seed (Figure 2(c)). All quantified polyphenols have clinical uses, i.e., rutin has a significant anticancer, antioxidant, and anti-inflammatory effect [25] and antimicrobial properties [26]. Likewise, kaempferol and quercetin have antimicrobial properties [27], antioxidant, and cytotoxic potential [28]. It can be concluded that C. ambrosioides seeds have a remarkable antimicrobial, antioxidant, and cytotoxic potential. The current evaluation may be due to the detection and quantification of polyphenols such as kaempferol, quercetin, and rutin.

Free radicals produced by the body are important biological substances. These reactive oxygen species (hydroxyl radical, hydrogen peroxide, superoxide radical, peroxyl, and peroxynitrite) are expressed more, cause oxidative stress, and are associated with the etiology of some diseases [29]. The determination of antioxidant potential using different methods has been used. Each test shows different antioxidant mechanisms, such as the elimination of free radicals, the decomposition of peroxides, and the prevention of chain initiation.

4.5. Free Radical Scavenging (DPPH) Assay. A total of 12 extracts exhibited ≥50% scavenging at 400 μg/ml concentration (Figure 3; Table 2). Me extracts showed the highest potential for DPPH bleaching, i.e., $80.12 \pm 1.53\%$ (IC_{50} 110.7 μg/ml). The free radical testing of DPPH is a simple, fast, reliable, cost-effective, and convenient way of studying the antioxidant properties of plant extracts [30]. 2, 2-Diphenyl-1-picrylhydrazyl, known as DPPH radical (molecular formula $C_{18}H_{12}N_5O_6$) is a cell permeable stable free radical. The delocalization of spare electron over the entire molecule does not allow the molecule to dimerise. The mechanism is based on the antioxidant ability to decolorize DPPH by forming yellow-colored diphenyl picrylhydrazine. ($DPPH_2$) results in quantitative measurement of the change in the absorbance value [31]. The results indicate that seeds may be a good source of antioxidants.

4.6. Total Antioxidant Capacity Determination. The total antioxidant capacity of crude extracts is estimated using phosphate molybdenum-based methods. The Me extract has the highest antioxidant capacity, that is, $110.6 \pm 2.2\,\mu$g AAE/mg (Figure 4; Table 2). NH extract revealed lowest antioxidant potential (11.2 ± 0.7 AAE/mg) among all the seed extracts. The phosphomolybdenum based total determination of antioxidant capacity relied on the conversion of MO (VI) to MO (V), which results in green-colored phosphomolybdate complex at acidic pH. This change of colour is noted by spectrophotometer and intensity of colour signifies antioxidant capability [32]. A human cell has been attacked by approximately 10,000 oxidative hits per day from reactive oxygen species that induce alteration of genetic damage which is an initial step towards development of mutagenesis [33]. The emergence of reactive oxygen species is an important factor in pathological problems such as protein oxidation, initiation of mutation of DNA, lipid peroxidation, and cellular degeneration leading to diabetes, cancer, Parkinson's, inflammatory, Alzheimer's, and cardiovascular diseases [29]. Natural antioxidants have been in continuous use for many years. Phytochemicals owing to antioxidant properties prevent development of degenerative diseases [34]. The presence of antioxidant capacity in the test extracts is attributed to the detection and quantification of phytochemicals in the analysis of TPC, TFC, and RP-HPLC in the present study.

4.7. Total Reducing Power Assay. Me ± extract exhibited maximum reducing power, i.e., $94.3 \pm 0.6\,\mu$g AAE/mg extract (Figure 4; Table 2). On the other hand, lowest

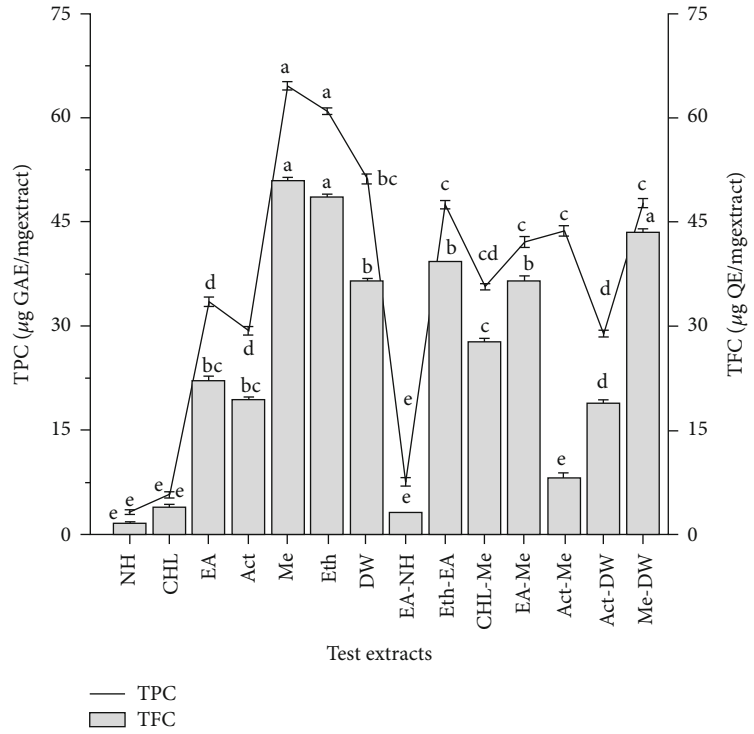

Figure 1: Total phenolics content (TPC) and total flavonoids content (TFC) determination in seed extracts of C. ambrosioides. Values are presented as mean ± standard error after triplicate analysis. (a)–(e) represent the significance of results. Samples that display different alphabets were significantly different at $p < 0.05$.

Table 2: Phytochemical analysis and antioxidant potential of different solvent extracts of C. ambrosioides.

No.	Extracts	Total phenolics content	Total flavonoids content	Free radical scavenging		Total antioxidant capacity	Total reducing power
		μg/mg	μg/mg	% inhibition	IC_{50} (μg/ml)	μg/mg	μg/mg
1	NH	3.2 ± 0.3^{e}	1.5 ± 0.3^{e}	$10.38 \pm 0.78_{e}$	—	11.2 ± 0.7^{e}	13.7 ± 0.8^{e}
2	CHL	5.7 ± 0.4^{e}	3.9 ± 0.4^{e}	$18.53 \pm 1.21_{e}$	—	32.5 ± 0.8^{e}	15.4 ± 0.7^{e}
3	EA	33.5 ± 0.7^{d}	22.3 ± 0.5^{bc}	60 ± 1.27^{b}	329 ± 5.0^{e}	50.6 ± 2.5^{d}	45.2 ± 0.9^{c}
4	Act	29.3 ± 0.6^{d}	19.5 ± 0.3^{bc}	59 ± 1.53^{b}	332 ± 3.0^{e}	35.6 ± 2.1^{d}	33.9 ± 0.9^{c}
5	Me	64.6 ± 0.6^{a}	50.9 ± 0.5^{a}	$80.12 \pm 1.53_{a}$	110.7 ± 5.0^{a}	110.6 ± 2.2^{a}	94.3 ± 0.6^{a}
6	Eth	60.9 ± 0.5^{a}	48.6 ± 0.4^{a}	78.6 ± 1.21^{a}	198 ± 4.0^{b}	99.7 ± 2.2^{a}	89.9 ± 0.9^{a}
7	DW	51.2 ± 0.7^{bc}	36.5 ± 0.3^{b}	56 ± 1.21^{b}	278 ± 3.0^{c}	71.8 ± 2.4^{b}	68 ± 0.2^{b}
8	EA-NH	7.6 ± 0.6^{e}	3.2 ± 0.3^{e}	52 ± 0.68^{b}	380 ± 3.0^{e}	37.3 ± 2.3^{e}	26.5 ± 0.2^{d}
9	Eth-EA	47.5 ± 0.6^{c}	39.4 ± 0.2^{b}	$68.31 \pm 1.21_{b}$	218 ± 2.0^{b}	87.8 ± 2.2^{b}	80.2 ± 0.9^{a}
10	CHL-Me	35.7 ± 0.4^{cd}	27.7 ± 0.4^{c}	$65.38 \pm 1.21_{b}$	294 ± 4.0^{c}	65.9 ± 2.4^{b}	61.9 ± 0.2^{b}
11	EA-Me	42.1 ± 0.7^{c}	36.6 ± 0.7^{b}	67.2 ± 0.78^{b}	226 ± 7.0^{b}	68 ± 2.9^{b}	62.7 ± 0.7^{b}
12	Act-Me	43.7 ± 0.7^{c}	8.2 ± 0.6^{e}	$57.6 \pm 1.08_{bc}$	360 ± 6.0^{e}	38.2 ± 2.7^{e}	33.3 ± 0.8^{c}
13	Act-DW	28.9 ± 0.5^{d}	19 ± 0.4^{d}	61 ± 0.51^{b}	308 ± 4.0^{e}	51.5 ± 2.1^{d}	45.6 ± 0.7^{c}
14	Me-DW	47.7 ± 0.6^{c}	43.4 ± 0.7^{a}	71 ± 0.49^{a}	200 ± 7.0^{b}	87.7 ± 1.7^{b}	78.2 ± 0.5^{b}

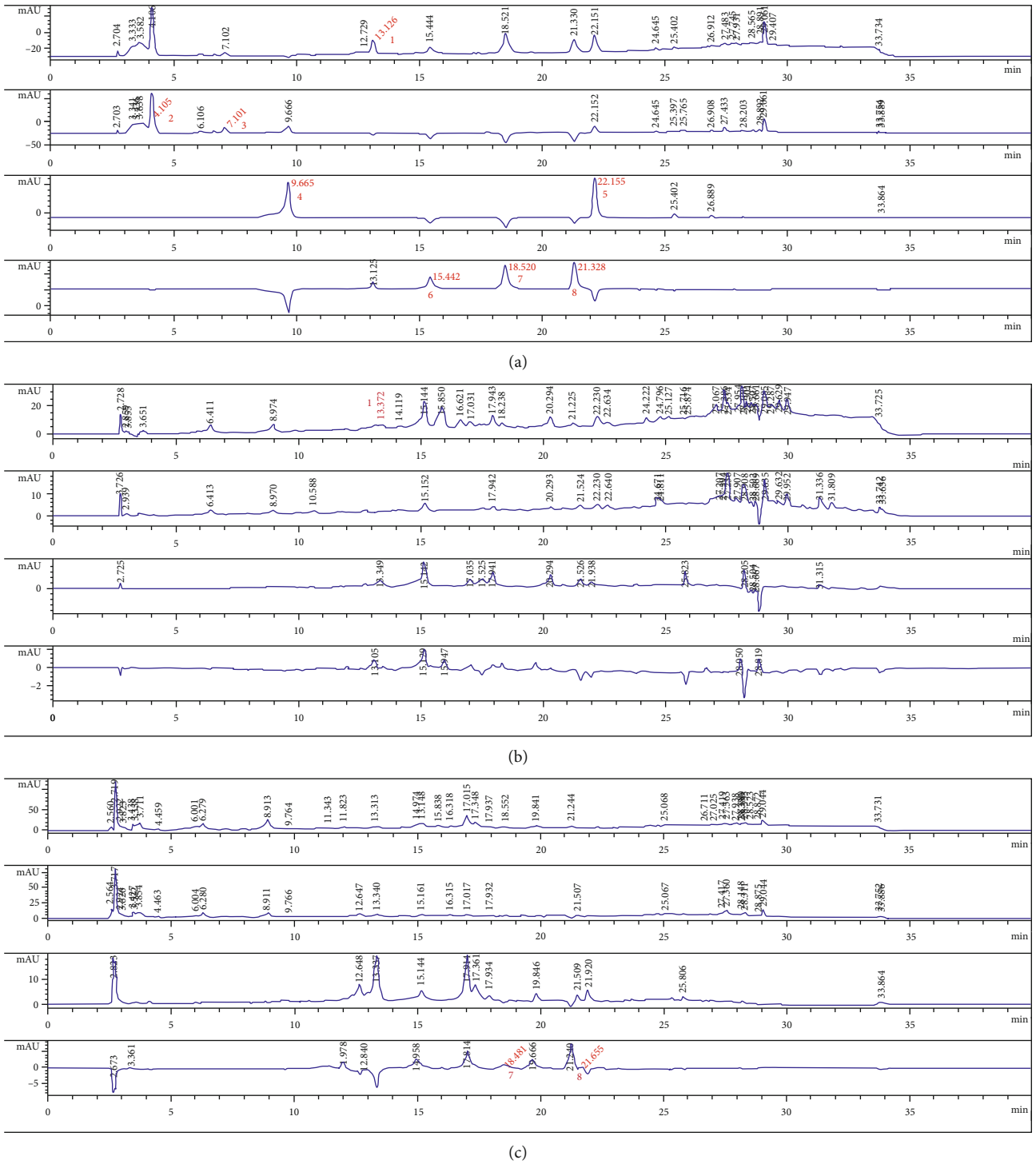

Figure 2: HPLC chromatograms of (a) standards, (b) Me extract of seed, and (c) Me-Dw extract of seed. 1; rutin. 2; gallic acid. 3; catechin. 4; caffeic acid. 5; apigenin. 6; myricetin. 7; quercetin. 8; kaempferol.

reduction potential was observed as $13.7 \pm 0.9\,\mu$g AAE/mg in NH extract. Reductones are thought to have caused the ability to exercise antioxidant effects by giving hydrogen atoms that cause chains of free radicals to break down [16]. Plants produce specific bioactive molecules that make them very effective antioxidants due to their strong H-producing capacity [35]. Assay findings further strengthen the antioxidant potential of C. ambrosioides seeds by indicating the presence of reductones.

4.8. Antimicrobial Assays. Medical research on infectious disease control is developing rapidly. However, drug abuse and resistance to antimicrobials still develop, and worldwide dispersion necessitates the development of advanced

Table 3: HPLC-DAD analysis of different solvent extracts of C. ambrosioides using standard polyphenols.

Extract name	Polyphenols (μg/mg extract)							
	GA	Rutin	CA	Catechin	AP Seed	Myrecetin	Quercetin	Kaemp
Me	—	1.98	—	—	—	—	—	—
Me-Dw	—	—	—	—	—	—	0.322	2.86

–: not detected; GA: gallic acid; CA: caffeic acid; AP: apigenin; Kaemp: kaempferol.

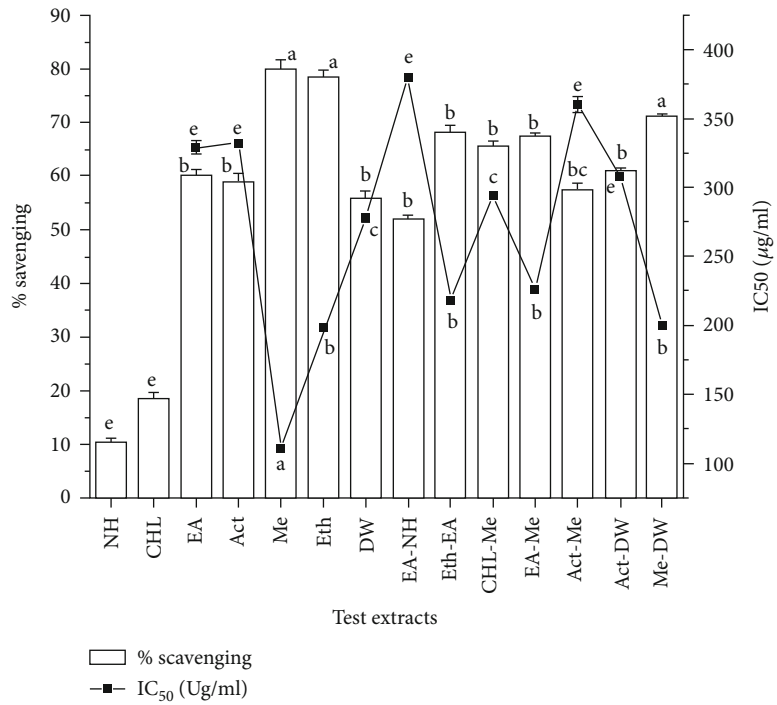

Figure 3: The determination of the percentage of free radical scavenging activity and the IC_{50} values of various solvent extracts in the seeds of C. ambrosioides. The values are presented as the mean average after triplicate experiment. (a)–(e) represent the significance of results. Samples that display different alphabets were significantly different at $p < 0.05$.

scientific approaches to establish novel therapeutic remedies [36]. Almost, all extracts have shown lower to moderate antibacterial potential. Maximum antibacterial activity was depicted when NH was employed to extract phytoconstituents (Table 4). Standard drug cefixime exhibited significantly higher activity against S. aureus, M. luteus, and K. pneumonia with 16 ± 0.7, 25 ± 1.04, 18 ± 0.81, and 17 ± 1.12 mm ZOI, respectively. The lack of growth inhibition areas around the impregnated discs confirmed the nontoxic effects of DMSO. The antimicrobial effect of active samples is difficult to attribute to more than one active principle because the different chemical compositions of each extract make it difficult to recognize. In addition to the main ingredients, minor components can also play an important role in extract biological activity [37]. It is possible that multiple botanical chemicals and essential oils present in plants produce synergistic or antagonistic effects [11]. Phenolic compounds might be responsible for obvious antimicrobial activity. Their antibacterial actions include affecting the function of the cytoplasmic membrane, disturbing the metabolism of energy, and affecting the synthesis of nucleic acids [38]. HPLC-based detection of rutin, kaempferol, and quercetin may be considered to be responsible for the current antibacterial activity being investigated.

All crude extracts were also tested for antimicrobial potential against five filamentous fungi strains. The extract showed that the test strain had a significant growth inhibition zone (Table 5).

The extracts of CHL and Act-Me seeds showed the greatest activity against F. solani and A. fumigatus with 17 ± 1.38 and 12 ± 1.42 mm ZOI, respectively (Table 5). Mucor specie and A. flavus strain were sensitive to EA-Me and Dw extracts with 14 ± 1.21 and 10 ± 0.78 mm ZOI, respectively. Maximum activity against A. niger was shown by Act-Me (15 ± 1.24 mm ZOI) extract. Standard drug clotrimazole (10 μg/disc) exhibited maximum activity. The lack of a growth inhibition zone around a DMSO-impregnated disc confirms the nontoxic effect of DMSO. The results are in accordance with the antifungal activity of essential oil obtained through hydrodistillation from aerial parts of C.

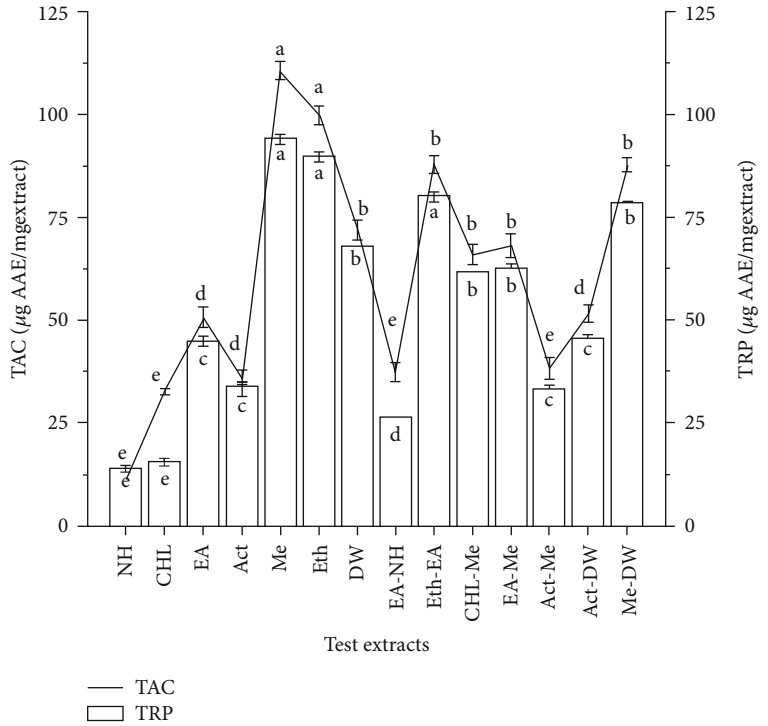

Figure 4: The total determination of antioxidant capability and reduction power in C. ambrosioides seed extracts. After a triplicate investigation, the values are displayed as mean ± average. (a)–(e) represent the significance of results. Samples that display different alphabets were significantly different at $p < 0.05$.

Table 4: Antibacterial activity of C. ambrosioides seed extracts against pathogenic bacteria.

Extract name	Zone of inhibition (mm) at 100 μg/disc		
	K. pneumoniae	M. luteus	S. aureus
NH	14 ± 1.61^{ab}	13 ± 1.30^{b}	9 ± 0.79^{b}
CHL	13 ± 1.39^{ab}	10 ± 0.97^{bc}	8 ± 0.69^{c}
EA	12 ± 1.27^{b}	10 ± 0.83^{bc}	8 ± 0.63^{c}
Act	13 ± 1.23^{ab}	8 ± 0.55^{d}	7 ± 0.55^{cd}
Me	7 ± 0.95^{e}	10 ± 0.71^{c}	8 ± 0.49^{c}
Eth	6 ± 0.79^{e}	9 ± 0.59^{cd}	8 ± 0.43^{c}
Dw	6 ± 0.83^{e}	8 ± 0.45^{d}	8 ± 0.39^{c}
EA-NH	9 ± 0.98^{d}	9 ± 0.81^{cd}	9 ± 0.67^{b}
CHL-Me	11 ± 0.81^{c}	10 ± 0.78^{c}	8 ± 0.61^{b}
EA-Me	8 ± 0.68^{d}	11 ± 0.98^{c}	9 ± 0.71^{b}
Eth-EA	10 ± 1.07^{cd}	11 ± 1.08^{c}	9 ± 0.81^{b}
Act-Me	12 ± 1.12^{c}	—	9 ± 0.73^{b}
Act-Dw	11 ± 1.11^{c}	7 ± 0.47^{d}	8 ± 0.53^{c}
Me-Dw	9 ± 0.61^{d}	8 ± 0.63^{d}	9 ± 0.83^{b}
Cefixime	17 ± 1.12^{a}	25 ± 1.04^{a}	16 ± 0.7^{a}
DMSO	—	—	—

Values are represented as mean ± standard error of triplicate experiments. –: no activity. Concentration of sample: 100 μg per disc. (a)–(e) represent the significance of results. Samples that display different alphabets were significantly different at $p < 0.05$.

ambrosioides [11, 39]. Different mechanisms are considered responsible for the antifungal effects of biomolecules, i.e., inhibition of cell wall formation, cell membrane disruption, mitochondrial dysfunction, and inhibition of cell division [40].

4.9. Brine Shrimp Cytotoxicity Assay. The cytotoxicity analysis of shrimp provides an effective preproposal method for predicting antimalarial, antimicrobial, insecticidal, and antitumor activities. Different solvent extracts of C. ambrosioides seeds showed cytotoxicity to brine shrimps on 24-hour exposure period in a concentration-dependent manner. The analysis revealed that brine shrimp cytotoxic activity with the lowest LC_{50} was shown by EA and CHL-Me extracts, i.e., 125 and 130 μg/ml, respectively (Table 6). The remaining extracts revealed their cytotoxicity in the following order: CHL > Act – Me > NH > EA – NH > Act > Eth – EA > Eth > Me > EA – Me > Act – Dw. Plant extracts with an LC_{50} of less than 1000 μg/ml were considered toxic to cells [16]. Most samples have LC_{50} values below 1000 μg/ml which represents a prominent cytotoxicity profile of crude extracts of C. ambrosioides seeds. The cytotoxicity observed in raw extracts may be due to the presence of secondary metabolites like phenols, flavonoids, terpenes [41], and other constituents which are not detected by HPLC analysis. This preliminary screening warrants detection of further polyphenols followed by isolation of potent compounds.

4.10. Protein Kinase Inhibition Potential. Protein kinase inhibitors are unique compounds that are specially characterized for inhibiting oncogenic kinase [42]. Protein kinase

Table 5: The antifungal activity of different solvents extracts of seeds of C. ambrosioides.

Extract name	Zone of inhibition (mm) at 100 μg/disc				
	F. solani	A. fumigatus	Mucor spp.	A. flavus	A. niger
NH	—	—	7 ± 0.43^{d}	7 ± 0.44^{c}	7 ± 0.39^{e}
CHL	17 ± 1.38^{b}	8 ± 0.51^{c}	10 ± 0.87^{bc}	—	9 ± 0.64^{cd}
EA	—	7 ± 0.31^{c}	12 ± 0.97^{b}	—	7 ± 0.25
Act	—	—	13 ± 1.13^{b}	7 ± 0.29^{c}	13 ± 1.21^{bc}
Me	8 ± 0.46^{c}	9 ± 0.67^{c}	—	—	13 ± 1.05^{bc}
Eth	8 ± 0.52^{c}	—	7 ± 0.39^{d}	—	9 ± 0.58^{cd}
Dw	—	8 ± 0.59^{c}	—	10 ± 0.78^{b}	7 ± 0.37^{e}
EA-NH	9 ± 0.63^{c}	—	8 ± 0.46^{d}	8 ± 0.61^{bc}	8 ± 0.49^{d}
CHL-Me	—	—	12 ± 1.13^{b}	8 ± 0.53^{bc}	8 ± 0.53^{d}
EA-Me	9 ± 0.69^{c}	12 ± 1.42^{bc}	14 ± 1.21^{b}	7 ± 0.39^{c}	11 ± 0.93^{c}
Eth-EA	8 ± 0.49^{c}	—	10 ± 0.87^{bc}	—	10 ± 0.97^{c}
Act-Me	9 ± 0.63^{c}	7 ± 0.47^{c}	13 ± 1.23^{b}	7 ± 0.37^{c}	15 ± 1.24^{b}
Act-Dw	9 ± 0.55^{c}	—	9 ± 0.59^{c}	—	7 ± 0.31^{e}
Me-Dw	—	8 ± 0.81^{c}	12 ± 0.93^{b}	9 ± 0.71^{b}	7 ± 0.42^{e}
Clotrimazole	30 ± 1.54^{a}	31 ± 0.9^{a}	30 ± 1.2^{a}	29 ± 0.5^{a}	31 ± 0.8^{a}
DMSO	—	—	—	—	—

Values are represented as mean ± standard error of three separate experiments. –: no activity. Clotrimazole (10 μg/disc) was positive control. (a)–(e) represent the significance of results. Samples that display different alphabets were significantly different at $p < 0.05$.

Table 6: Cytotoxic potential of different solvent extracts of C. ambrosioides seed.

Extract name	Brine shrimp lethality		Protein kinase inhibition	
	% mortality 1000 μg/ml	LC_{50} μg/ml	Diameter (mm) at 100 μg/disc Clear zone	Bald zone
NH	100	152^{bc}	—	11 ± 0.83^{b}
CHL	100	133^{b}	—	10 ± 0.49^{b}
EA	97.5	125^{b}	—	10 ± 0.61^{b}
Act	93	179^{bc}	—	12 ± 0.91^{b}
Me	100	250^{c}	—	20 ± 1.34^{a}
Eth	97.5	228^{c}	—	20 ± 1.27^{a}
Dw	35	$>1000^{e}$	—	—
EA-NH	97	168	8 ± 0.68	—
CHL-Me	90	130^{b}	—	—
EA-Me	80	255^{c}	—	—
Eth-EA	97	185^{bc}	10 ± 0.78	—
Act-me	95	137^{b}	15 ± 1.08	—
Act-Dw	52	971^{d}	9 ± 0.51	—
Me-Dw	50	$>1000^{e}$	11 ± 0.49	—
Doxorubicin	—	5.93^{a}	—	—
Surfactin	—	—	—	21^{a}
DMSO	—	—	—	—
1% DMSO in water	—	—	—	—

The values are presented as an average mean with standard error after triple analysis. –: no activity or not applicable. (a)–(e) represent the significance of results. Samples that display different alphabets were significantly different at $p < 0.05$.

inhibitor (PKg2) in the sample blocks Streptomyces aero hyphal formation, thus suggesting that it inhibits the proliferation of cancerous cells. The results are summarized in Table 6. Among all seed extracts, only unisolvent extracts showed remarkably significant inhibition of the formation of hyphae at 100 μg/disc. The maximum activity of the Me and Eth extracts is shown, that is, 20 ± 1.34 and 20 ± 1.27 mm bald zone of inhibition, respectively (Table 5). The

nontoxic effect of DMSO was confirmed by the absence of ZOI. Positive control surfactin (20 μg/disc) showed 21 mm ZOI. The abnormal activity of protein kinases is overexpression, mutation, or deregulation, avoiding physiological processes and causing life-threatening cancers. The ability of samples and extracts to bind to the active or inactive sites of a kinase as a whole is crucial for the development of new drugs to prevent chemotherapy. The results are consistent with previous reports in which extracts of Me from various sources show an anti-inflammatory reaction by directly inhibiting several interleukins-1-receptor and mTOR kinases [43, 44]. The activity exhibited by Me-Dw extract of C. ambrosioides is believed to inhibit cancer linked several kinases (ABL1, CLK1, MET, and NEK4), and the existence of quercetin (detectable by HPLC fingerprints) is attributed to this activity [45]. In this study, various extracts show significant inhibition potentials of kinases, which may be a powerful source of chemoprevention drugs.

5. Conclusion

Extraction in wide polarity solvents is crucial to determine therapeutic potential. Plant biochemicals are dependent on the polarity of extraction solvents. The quantification of RP-HPLC (rutin, quercetin, and kaempferol), as well as the good antioxidant potential, makes the seeds of C. ambrosioides a good source of antioxidants. Significant results were obtained by extracts in antibacterial, antifungal, cytotoxic salt shrimp, and protein kinase inhibition studies. The optimal solvent system should be used for extraction on a preparative scale to improve productivity. The current study also demonstrates promising potential of C. ambrosioides seeds for the discovery of novel bioactive moieties through bioactivity guided isolation.

Data Availability

All the data is original based on extensive research and can be provided as supplementary data if required.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

S.B. performed experiments, analyzed and interpreted the data, did software statistics, and wrote and revised the manuscript. M.W.B. and S.S.Z. assisted in in vitro experiments, acquisition of the data, and critical review of the manuscript. M.K.O. and N.A. assisted in data analysis and interpretation and made critical revisions. W.H.A.-Q. and H.A. contributed to the acquisition of the data and critical review of the manuscript. I.-u.H. conceptualized and designed the study, supervised execution of experiments, critically revised the manuscript, and approved the final version of this manuscript. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

The authors extend their appreciation to the researchers supporting project no (RSP-2021/293) at King Saud University, Riyadh, Saudi Arabia. The authors are thankful to Prof. Dr. Rizwana Aleem Qureshi, Department of Plant sciences, Faculty of Biological sciences, Quaid-i-Azam University Islamabad, Pakistan, for identifying the plant sample. University Research Fund from Quaid-i-Azam University Islamabad is acknowledged for execution of the study.

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