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Research Article

Antimicrobial and Antioxidant Activity of Secondary Metabolites Isolated from *Citrullus colocynthis* (L.) Schrad.

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Abstract: Available antibiotics have lost their efficiency against several multidrug-resistant (MDR) microbes. Phytochemicals possess great antimicrobial activity and can be an alternative to available antibiotics for MDR microbes. *Citrullus colocynthis* (L.) Schrad is reported as an antimicrobial and anticancer herb in traditional medicinal cultures. Column chromatography was used to isolate secondary metabolites from ethanolic extracts of *C. colocynthis* whole fruits. Agar well diffusion method was used to determine antimicrobial activity. Antioxidant activity was measured by the DPPH (1,1-Diphenyl-2-Picrylhydrazine) radical scavenging assay. In this research, the informant consensus factor (ICF) and fidelity level (FL) were calculated on the basis of data collected from local herbalists and elderly villagers of age groups 51–60 who had knowledge of ethnomedicinal uses of plants. It was found that mostly the fruit and its parts (rind, pulp, and seeds) of *C. colocynthis* were used for the treatment of cancer and microbial infections. Alkaloids showed significant antibacterial activity against *Micrococcus luteus* (activity index 1.11; zone of inhibition (29.1±0.3 mm) *and Pseudomonas pickettii* (activity index 1.14; zone of inhibition 32.4±1.7 mm) as compared to streptomycin. It was noticed that flavonoids and phenolics at a concentration of 2000 μLmL^{-1} showed significant inhibition of free radicals, i.e., 91.57 % and 92.31 %, respectively. It was slightly higher than that of standard butylated hydroxytoluene (BHT), which was 89.07 %. It was found that with the increase in the concentration of phytochemicals, their radical scavenging potential also increased. It can be concluded that alkaloids are the main antimicrobial agents. Flavonoids and phenolics have great potential for free radical scavenging.

Keywords: Antibacterial, Antifungal, Antimicrobial, Antioxidant, *Citrullus colocynthis,* Fidelity Level, Informant's Consensus Factor, Plant Secondary Metabolites.

1. INTRODUCTION

Numerous antimicrobial formulae have lost their effectiveness against a number of pathogenic bacteria. These multidrug-resistant pathogenic bacteria are wreaking havoc on people's health and credit. Natural medicines are increasingly popular these days [1]. Because of multi-drug resistance, the death rate and loss of credit have increased [2]. Multidrug resistance is now a global hazard to public health. Several microbes, including *Clostridium difficile, Neisseria gonorrhoeae*, *Candida albicans*, *Pseudomonas aeruginosa, Salmonella typhimurium, Streptococcus pneumonia*, and *Staphylococcus aureus,* have become resistant to available antibiotics [3]. The threat is greater in underdeveloped nations, where the prevalence of contagious diseases is considerable and the emergence of antibiotic resistance is also present [4]. Treatment of bacterial infections has become more expensive and less effective due to these resistant strains of bacteria [5]. The presence of multidrug-resistant microbes in humans and domestic animals has been documented in research [6]. The majority of foodborne pathogens are resistant to antibiotics, and the fundamentals of this resistance are not understood. There is a need to find new ways of treating multi drug resistant bacteria [7].

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Plants have long been used as herbal remedies for a large number of diseases and disorders. All the traditional medicinal cultures have greatly relied on plants for safe, easy, and affordable medical remedies. Therapeutic agents obtained from medicinal plants are thought to be a possible alternative to available antibiotics [8]. These alternative medicines can be used to overcome the problems of antibiotic resistance and loss of credit [9]. Plant-oriented medicines are getting more popular with increasing drug resistance in common human pathogens [10]. *C. colocynthis* seed extracts possess antimicrobial, radical scavenging, and antiproliferative potential [11]. Secondary metabolites obtained from plants can be possible alternatives to antibiotics. Plant-oriented medicines have fewer side effects and greater efficiency [12]. In the past, people used to rely on natural herbs and plant parts as medicines [13].

Citrullus colocynthis L. Schrad is a medicinal plant that belongs to the Cucurbitaceae family. It is a xerophytic herb [14]. It possesses antimicrobial, antioxidant, anticancer, and hypolipidemic potential [15]. *C. colocynthis* possesses sufficient amounts of antioxidants and phenolic contents in fruit, peel, pulp, and seeds [16]. Triterpenoid spinasterol and 22,23-dihydrospinasterol isolated from *C. colocynthis* leaf extracts showed great antifungal, antioxidant, and aphicidal activity [17]. Cucurbitacin B from *Ecballium elaterium* L. improved the antibacterial and antiviral activity of antibiotics used against some isolates of multidrug-resistant human pathogens [18]. Fernonol and 22,23-dihydrospinasterol isolated from *C. colocynthis* have shown significant pesticidal activity [19].

Natural substances are found in various types of organisms and exhibit a wide range of structures. Metabolites like tannins, anthocyanins, and alkaloids are unquestionably substances of great interest to the pharmaceutical industries for manufacturing different medicines [20]. Whole fruits and vegetables contain a variety of antioxidants in the form of secondary metabolites. These plant-oriented metabolites should be preferred to synthetic antioxidants. Regular consumption of synthetic metabolites causes additional complications in cardiovascular disease and cancer [21].

The goal of the current study was to identify plant secondary metabolites from *C. colocynthis* ethanolic fruit extracts and determine their bioactivity for possible antibacterial activity and antioxidant activity. Three gram-positive and three gram-negative bacterial strains were used to assess the antibacterial activity. Streptomycin and Griseofulvin two common antibiotics, were used to compare the effectiveness of antimicrobial action.

2. MATERIALS AND METHODS

2.1 Analysis of Traditional Ethno-Medicinal Importance

Data on the ethnomedicinal importance of *C. colocynthis* were collected from 118 elderly people from villages in district Bhimber, Azad Jammu, and Kashmir (AJ&K), Pakistan. Older people were selected because they have better knowledge of the traditional uses of medicinal plants. A questionnaire was prepared about the traditional importance of *C. colocynthis* and its parts being used traditionally for different ailments or disorders. From the gathered data, Fidelity Level was calculated by following Friedman *et al.* [22].

$$FL = N_p/N \times 100$$

Here "N = Total number of respondents" and "N_p = Number of respondents who reported specific use of particular plant".

For the measurement of Informant's Consensus Factor, following formula was followed [23].

$$ICF = n_{ur} - n_t/n_{ur} - 1$$

Where n_{ur} = Number of Uses of a particular plant against a particular disease reported by respondents.

n_t = Total number of plants used for treatment of this particular disease.

2.2 Sample Collection

Plants along with fruits were collected from Bhimber, 32° 28′ 0″ N (latitude) and 75° 6′ 0″ E (longitude), AJ&K, Pakistan. Plants were identified by a taxonomist. Identified *C. colocynthis* was submitted to the herbarium of the Department of

Botany under reference no. MUST-Bot.-MUH-517. Fruits were separated and shade-dried for 2 months.

2.3 Crude Extraction

Fruits were ground into a fine powder. The cold soaking method was used for crude extraction, following Preethi *et al.* [24]. Fruit powder (100 g) was soaked in 500 mL of ethanol (BDH, Poole, England Cat# 101077Y). Solute was kept soaking for 7 days. After 7 days of soaking, Whatman filter paper (no. 42) was used for filtration. A rotary evaporator (EYELA N1100, China) was used for the evaporation of the solvent. After evaporation of the solvent, the remaining crude extracts were stored at 4 ˚C for further experimentation.

2.4 Phytochemistry

2.4.1. Column chromatography (CC)

Separation of individual classes of compounds from crude fruit extracts was done by column chromatography following the published method of Ahmad *et al.* [19]. The stationary phase in CC was a silica gel (200–300 mesh) column. The mobile phase consisted of ethyl acetate (AE) and petroleum ether (PE) in four different proportions. These fractions were AE:PE, i.e., 25:100, 50:100, 75:100, and 100:100 mL. The fractions obtained through column chromatography were confirmed by the following procedure.

2.4.2. Confirmation of phytochemicals

Isolated fractions were identified by confirmation tests following Harborne *et al.* [25] for the confirmation of alkaloids, flavonoids, glycosides, saponins, and tannins. For the confirmation of phenolics and terpenoids method of Harith *et al.* [26] was followed.

2.5 Antimicrobial Activity

2.5.1. Pathogenic strains

Micrococcus luteus, Staphylococcus epidermitis, and *Listeria monocytogenes* were gram-positive pathogenic bacterial strains, *Pseudomonas pickettii*, *Vibrio cholera*, and *Vibrio parahaemolyticus* were gram-negative pathogenic bacterial strains and *Alternaria alternata, Botyritis cinera,* and *Curvularia lunata* were the fungal strains that were selected for the experiments. Test bacterial strains and fungal taxa were obtained from the Department of Botany, Mirpur University of Science and Technology (MUST), Mirpur, AJ&K, Pakistan.

2.5.2. Culture medium

Potato Dextrose Agar (PDA) medium with pH 5.6 was prepared for the growth of microbial strains following Mazher *et al.* [27]. PDA powder weighing 39 g was dissolved in 900 mL of distilled water and boiled until a uniform mixture of yellowish colour was obtained. After that volume of the solution was raised to 1L. PDA medium and all glassware were autoclaved for 15 min at 121 °C before using as culture medium for inoculation of bacterial or fungal strains.

2.5.3. Zone of inhibition

Agar well diffusion method was used for measurement of zone of inhibition following Perez *et al,* [28]. Petri plates were placed in laminar flow to avoid contamination. In each plate wells measuring 5 mm were made through cork borer. Streptomycin and isolated phytochemicals were poured into these wells with the help of a micropipette. Streak method was used for inoculation of pathogenic strains. Streaked petri plates were placed in an incubator at 37 °C for 48 h. After that zone of inhibition (clear zone with no bacterial/fungal growth) was measured. Each experiment was performed in triplicate.

2.5.4. Determination of activity index (AI)

Antimicrobial activity index of extracts was measured by the following formula [29].

Activity Index = ZI (Sample) / ZI (Standard)

Here, ZI (Sample) means Zone of Inhibition shown by a particular sample ZI (Standard) means Zone of Inhibition shown by standard antibacterial/ antifungal drug.

2.6 Measurement of Antioxidant Activity

Antioxidant potential of phytochemicals isolated

from *C. colocynthis* fruit extracts was determined following Brand-Williams *et al.* [30]. For radical scavenging assay, 1,1- Diphenyl-Picrylhydrazine (DPPH) was used and its result were validated with standard antioxidants i.e. α-tocopherol, Ascorbic acid and BHT (butylated hydroxytoulene).

2.6.1. Preparation of stock solution and serial dilutions

Each isolated phytochemical weighing 0.02 g was dissolved in 10 mL of methanol. Final volume of the solution was raised to 20 mL. Solutions of 250 μLmL^{-1}, 500 μLmL^{-1}, 1000 μLmL^{-1} and 2000 μLmL^{-1} concentration were prepared from stock solution.

2.6.2. Preparation of DPPH solution

For DDPH Radical Scavenging Assay (DSRA) 33 mL of 0.01 mM solution of DPPH was dissolved in 1L of methanol.

2.6.3. DPPH radical scavenging assay (DSRA)

Free radical scavenging activity of different phytochemicals isolated from *C. colocynthis* was checked through DSRA following Shekhar and Anju [31]. For DSRA 1 mL of each stock solution was taken in different cuvettes and 5 mL of DPPH was added in each cuvette. These cuvettes were kept for 30 min at room temperature and then absorbance was determined at 515 nm. Results were compared with standard antioxidants. Free radical scavenging activity was determined by percentage inhibition by the following formula;

$$\% \text{ inhibition} = (A_C - A_P) / A_B \times 100$$

A_C = Absorbance of Control
A_P = Absorbance of Phytochemical
A_B = Absorbance of Blank

2.7 Statistical Analysis

All the data were analyzed through the statistical package for social sciences (SPSS 16.0) and results are presented as arithmetic mean ± standard deviation (SD). One-way ANOVA (analysis of Variances) was carried out for comparing means. Values $P < 0.05$ were deliberated statistically significant. Duncan multiple range test (DMRT) was carried out for the values that were significant

3. RESULTS

3.1 Ethnobotanical Importance of *C. colocynthis*

Result of ICF indicated that *C. colocynthis* has been in use traditionally for the treatment of several diseases or disorders. However, it is mostly used for antidiabetic and antilipidemic activities as per ICF of 13 %. ICF also indicates that it has been used for antimicrobial and antioxidant activities with ICF citation of 12 % and 11 % respectively as shown in figure 1.

The FL% results show that all parts of *C. colocynthis* have traditionally been used to treat various ailments (Figure 2). With an FL value of 23 %, whole fruit and seeds are the most important medicinal part of *C. colocynthis*. Fruit rind is the second-most important plant part, with a 21 % FL value. With an FL value of 20 % fruit pulp is the third most important parts of *C. colocynthis*. Most citations of using *C. colocynthis* fruits and parts were reported by local herbalists and elderly villagers, we studied the effectiveness of *C. colocynthis* fruit and validated the use of *C. colocynthis* fruit parts for antimicrobial and antioxidant activities in this study.

3.2 Column Chromatography for the Isolation of Different Class of Phytochemicals

Different confirmatory tests were performed for the identification of individual classes of compounds. Results of confirmatory tests are presented in Table 1. Nine different fractions were obtained by column chromatography. Different colored fractions were taken in different test tubes. Test tubes were labeled with capital alphabets from A to I as shown in Figure 3. For the confirmation of phytochemicals in each fraction, different phytochemical tests were performed.

Confirmatory experiments were performed three times, and finally the fractions obtained by column chromatography were confirmed as A and B being alkaloids, C being phelonics, D being glycosides, E being saponins, F being flavonoids, and H and I being terpenoids. Details of the

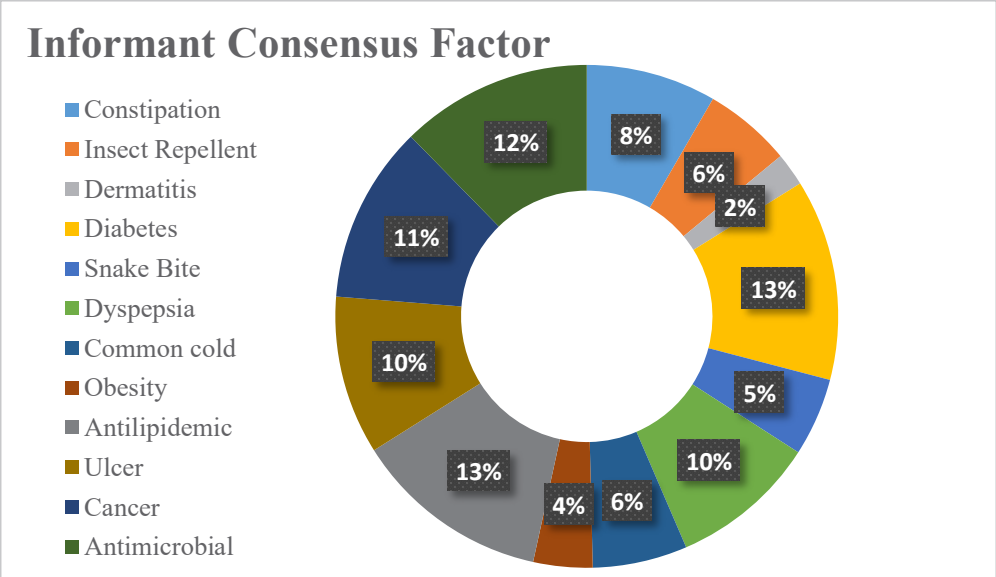

Fig. 1. Informant's Consensus Factor of *C. colocynthis* calculated on the basis of data gathered from local herbalists and elderly villagers

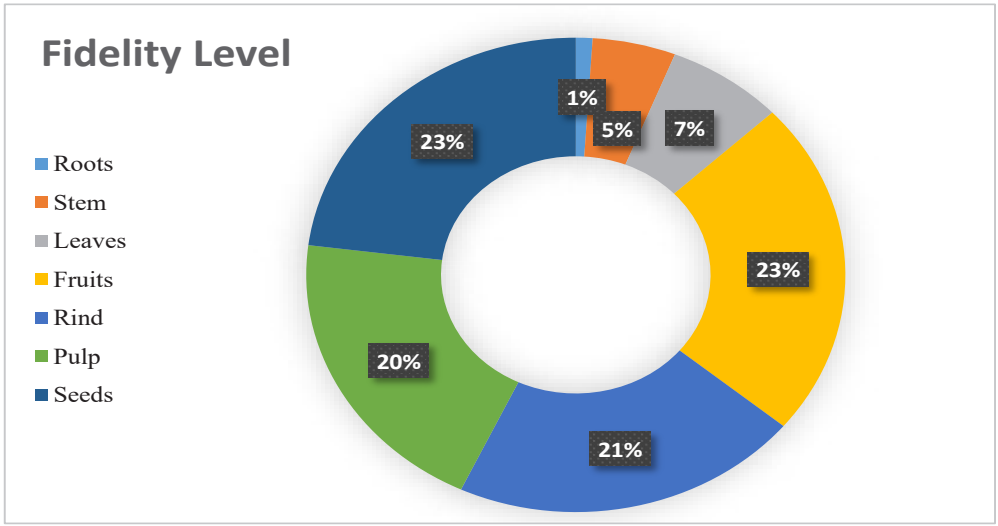

Fig. 2. Fidelity Levels of different parts of *C. colocynthis*

confirmative aspects are given in Table 1.

3.3. Antimicrobial Activity

3.3.1. Antibacterial activity of individual class of phytochemicals against gram-positive bacterial strains

Antibacterial activity of different phytochemicals was tested against three gram-positive bacterial strains, including *Listeria monocytogenes, Micrococcus luteus,* and *Staphylococcus epidermitis.* The activity of phytochemicals was compared with that of the standard antibiotic streptomycin. Alkaloids, flavonoids, phenolics, and terpenoids showed antibacterial activity; however, only alkaloids' antibacterial activity was significant as compared to the standard drug. Alkaloids have the highest zone of inhibition of 29.1 ± 0.3^{a} mm against Micrococcus luteus, which is significantly higher than streptomycin 26.3 ± 0.3^{b}, with an activity index of 1.11 (Table 2).

In one petri dish, only streptomycin was poured into a 5 mm agar well. Whereas in all other petri plates except the control, three wells were bored, and in

Fig. 3. Fractions obtained by column chromatography.

Table 1. Confirmatory tests for different fractions isolated through column chromatography

Phytochemicals	Tests Performed	Confirmative Aspect	Fraction(s)
Alkaloids	Dragandroff Test	Red precipitate formation	A, B
	Hager Test	Yellow colour formation	
	Mayer Test	Yellow colored precipitate	
	Wagner Test	Brown reddish precipitate	
Flavonoids	Ferric Chloride	Becomes colorless	F
	Alkaline Reagent	Yellow colour precipitate	
	Lead acetate Test	Orange to red coloration	
Glycoside	Bromine water Test	Brownish coloration	D
	Keller Test	Dark brown ring formation	
Phenolics	Ferric Chloride Test	Blue and green coloration	C
Saponins	Foam test	Appearance of froth	E
	Bromine water test	Blue-black coloration	
Tannins	Ferric chloride Test	Brown ring at the junction	G
Terpenoids	Salkowaski Test	Reddish brown coloration	H, I
	Liebermann Test	Appearance of froth	

each well, a different phytochemical was poured. Zones of inhibition were measured after 48 h. The zone of inhibition of alkaloids against *M. luteus* was significantly greater than that of streptomycin (Fig. 4). However, flavonoids and glycosides showed little antibacterial activity.

3.3.2. Antibacterial activity of individual class of phytochemicals against gram-negative bacterial strains

Glycosides, tannins, and saponins showed negligible antibacterial activity whereas flavonoids, phenolics, and terpenoids showed antibacterial activity but it was not significant as compared to standard antibiotic streptomycin (Table 3). Alkaloids showed significant antimicrobial activity as compared to streptomycin.

Alkaloids showed 32.4 ± 1.7^{a} mm zone of inhibition (ZI) against *Pseudomonas pickettii,* it was significantly greater than ZI of streptomycin i.e. 28.3 ± 1.3^{b} mm (Fig. 5). An activity Index of 1.14 was calculated for alkaloids against *P. pickettii.*

Table 2. Zone of Inhibition (ZI) and Activity Index (AI) of phytochemicals isolated from *C. colocynthis* crude extracts against gram positive bacterial strains

Phytochemical	*Listeria monocytogenes*		*Micrococcus luteus*		*Staphylococcus epidermitis*	
	ZI (mm)	**AI**	**ZI (mm)**	**AI**	**ZI (mm)**	**AI**
Alkaloids	24.5±0.7^{b}	0.89	29.1±0.3^{a}	1.11	22.9±1.3^{b}	0.89
Flavonoids	17.4±1.1^{c}	0.63	12.4±1.7^{e}	0.47	16.2±0.6^{c}	0.63
Glycosides	07.2±0.5^{f}	0.26	9.3±0.3^{f}	0.32	09.2±0.5^{e}	0.36
Phenolics	13.3±0.4^{d}	0.49	16.1±0.6^{c}	0.61	11.3±0.3^{d}	0.44
Saponins	NG	NG	NG	NG	NG	NG
Tannins	NG	NG	NG	NG	08.1±0.3^{f}	0.32
Terpenoids	11.7±2.0^{e}	0.43	14.4±2.8^{d}	0.55	16.0±1.0^{c}	0.62
Streptomycin	27.4±0.7^{a}		26.3±0.3^{b}		25.7±0.7^{a}	

Values are expressed as Mean±SD (n = 3). Level of significance 95% (P<0.05). Different superscripts indicate significant differences; NG = Not Given.

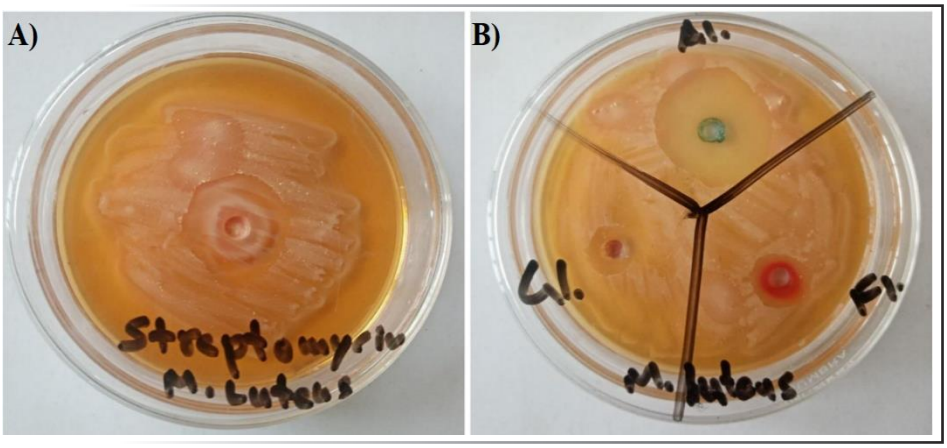

Fig. 4. Zone of inhibition against *Micrococcus luteus* shown by streptomycin **(A)** and different phytochemicals **(B)**. Al. = Alkaloids; Fl. = Flavonoids; Gl. = Glycosides

Table 3. Zone of Inhibition (ZI) and Activity Index (AI) of phytochemicals isolated from *C. colocynthis* crude extracts against gram-negative bacterial strains

Phytochemical	**Pseudomonas pickettii**		**Vibrio cholera**		**Vibrio parahaemolyticus**	
	ZI (mm)	**AI**	**ZI (mm)**	**AI**	**ZI (mm)**	**AI**
Alkaloids	32.4±1.7^{a}	1.14	30.1±0.7^{a}	0.98	25.3±2.7^{b}	0.92
Flavonoids	17.6±1.2^{c}	0.62	16.7±0.3^{c}	0.54	14.2±0.5^{c}	0.52
Glycosides	NG	NG	10.2±1.4^{e}	0.30	09.0±1.1^{e}	0.33
Phenolics	14.1±0.7^{d}	0.50	17.3±0.7^{b}	0.56	14.2±0.8^{c}	0.52
Saponins	08.7±3.3^{e}	0.31	NG	NG	11.4±2.3	0.41
Tannins	NG	NG	NG	NG	NG	NG
Terpenoids	14.3±0.7^{d}	0.51	12.5±0.5^{d}	0.41	13.6±1.3^{d}	0.49
Streptomycin	28.3±1.3^{b}		30.7±0.6^{a}		27.5±0.6^{a}	

Values are expressed as Mean±SD (n = 3). Level of significance 95% (P<0.05). Different superscripts indicate significant difference; NG = Not Given

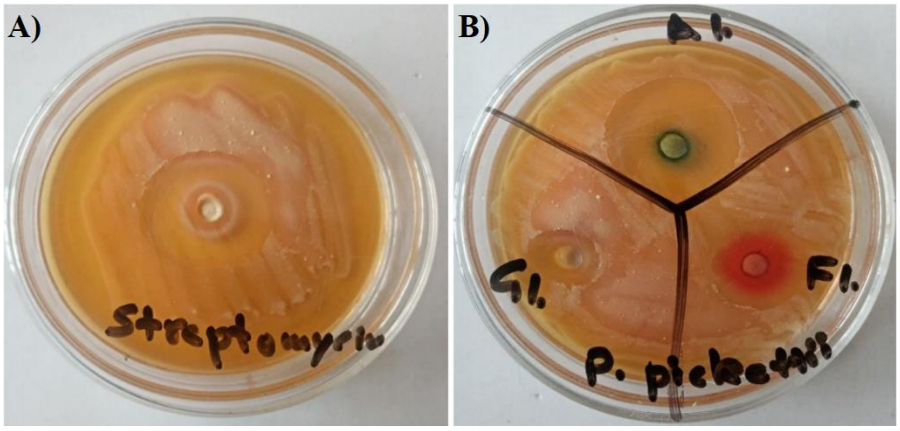

Fig. 5. Zone of inhibition against *Pseudomonas pickettii* shown by streptomycin **(A)** and different phytochemicals **(B)**. Al.= Alkaloids; Fl.= Flavonoids; Gl.= Glycosides

3.3.3. Antifungal activity of individual class of phytochemicals

It was noted that tannins showed no antifungal activity, whereas saponins and glycosides showed negligible antifungal activity. Flavonoids, phenolics, and terpenoids showed antifungal activity, but it was not significant as compared to standard Griseofulvin. The antifungal activity of alkaloids was comparable to that of griseofulvin but not higher (Table 4).

3.4 Antioxidant Activity of Individual Class of Phytochemicals

It was shown that as phytochemical concentrations increased, so did their radical scavenging potential. It was noted that flavonoids and phenolics at a concentration of 2000 μLmL^{-1} showed significant inhibition of free radicals, i.e., 91.57 % and 92.31 %, respectively. It was slightly higher than the industry standard of 89.07 %. Alkaloids, glycosides, and saponins also showed comparable antioxidant activity, whereas tannins and terpenoids showed negligible antioxidant activity (Table 5). Values are expressed as Mean±SD (n = 3). Level of significance 95 % (P<0.05). Star as a superscript indicates higher antioxidant activity as compared to standard antioxidants

4. DISCUSSION

Present study has found that *C. colocynthis* is mostly employed for antidiabetic, antilipidemic,

Table 4. Zone of Inhibition (ZI) and Activity Index (AI) of phytochemicals isolated from *C. colocynthis* crude extracts against pathogenic fungal strains

Phytochemical	*Alternaria alternata*		*Botyritis cinera*		*Curvularia lunata*	
	ZI (mm)	**AI**	**ZI (mm)**	**AI**	**ZI (mm)**	**AI**
Alkaloids	29.0±1.3^{b}	0.86	28.7±1.6^{b}	0.93	22.4±0.6^{b}	0.77
Flavonoids	20.3±3.7^{c}	0.61	16.2±0.7^{c}	0.52	17.7±1.3^{c}	0.61
Glycosides	11.0±1.3^{f}	0.33	09.7±3.3^{e}	0.31	NG	NG
Phenolics	11.2±0.1^{f}	0.33	10.3±0.5^{e}	0.33	12.4±1.1^{e}	0.42
Saponins	13.6±1.6^{e}	0.41	NG	NG	NG	NG
Tannins	NG	NG	NG	NG	NG	NG
Terpenoids	17.5±1.3^{d}	0.52	12.3±0.3^{d}	0.40	13.9±1.3^{d}	0.48
Griseofulvin	33.7±0.7^{a}		30.9±1.3^{a}		29.2±0.6^{a}	

Values are expressed as Mean±SD (n = 3). Level of significance 95 % (P<0.05). Different superscripts indicate significant difference; NG = Not Given

Table 5. DPPH Radical Scavenging Activity (% inhibition) of phytochemicals from *C. colocynthis* at different concentrations

Phytochemical	Concentration			
	250 µl/ml	500 µl/ml	1000 µl/ml	2000 µl/ml
Alkaloids	56.83±2.7	61.62±1.1	65.41±3.6	66.88±4.5
Flavonoids	60.23±3.5	77.93±2.7	89.74±0.4*	91.57±4.9*
Glycosides	55.71±2.2	59.13±5.4	66.87±4.5	70.03±5.5
Phenolics	73.03±7.4	84.55±3.4	86.63±7.1	92.31±1.7*
Saponins	70.13±4.2	75.44±5.1	81.20±3.2	84.34±1.6
Tannins	23.11±4.4	24.55±3.4	29.60±2.3	37.41±1.1
Terpenoids	20.24±3.6	26.57±6.3	34.30±0.3	41.12±3.7
Standards			2000 µl/ml	
BHT			89.07±4.7	
Ascorbic Acid			81.21±1.7	
α-tocopherol			87.59±5.2	

antimicrobial, and antioxidant activity. All parts of the *C. colocynthis* have historically been used to treat a variety of diseases, according to the fidelity level (FL) percentage, but the fruit and all of its components, including the rind, pulp, and seeds, receive the most mentions. FL and ICF for *C. colocynthis* have not been documented yet. The current work has used ethanolic fruit extracts to separate several phytochemicals for researching antibacterial and antioxidant activity based on the findings of the ethnobotanical surveys (ICF and FL).

Many antibiotics no longer work as well against a variety of pathogens [32]. A large number of bioactive alkaloids and flavonoids are constituents of *C. colocynthis* and have antibiotic potential. Recently, a pesticide formulation (NNRC-82) has been developed from *C. colocynthis,* and its patent has been registered [33]. Alkaloids, flavonoids, phenolics, and terpenoids all exhibited antibacterial action, but alkaloids' antibacterial activity was comparable to that of prescription drugs. Alkaloids demonstrated higher zones of inhibition than streptomycin against *pickettii* and *Micrococcus luteus*. The findings of this study are consistent with those of earlier investigations by [34] in which ethanolic extracts of whole *C. colocynthis* were found to be highly antibiotic. The results of this study are also in accordance with [35-36], which revealed that alkaloids have strong antibacterial properties.

Numerous illnesses in humans and animals are brought on by fungus-borne infections. A few biochemically active plant chemicals that can thwart fungus growth have been identified by scientists [37]. This study examined the antifungal efficacy of isolated phytochemicals against *Botyritis cinera*, *Curvularia lunata*, and *Alternaria alternata* and compared it to the activity of Griseofulvin, a common antifungal medication. It was revealed that saponins and glycosides had minimal antifungal action compared to tannins, which exhibited no activity. The antifungal activity of flavonoids, phenolics, and terpenoids was not significant. Alkaloids demonstrated antifungal efficacy that was comparable to that of Griseofulvin. Previous studies [38, 39] investigated the antifungal activity of *C. colocynthis* extracts and found similar results. They have found that *C. colocynthis* seed extracts are significantly antifungal against *Aspergillus niger* and *A. flavus* but none of the zones of inhibition were greater than those of standard.

DNA damage and malignancies are mostly caused by reactive oxygen species (ROS). Antioxidants are consumed in various ways to treat these issues. Plant phytochemicals are confirmed by research to be very powerful antioxidants. In addition to removing ROS, phytochemicals are also less expensive and have fewer adverse side effects [19, 40]. In the present study, antioxidant activity of isolated phytochemicals was determined by the DPPH free radical scavenging assay (DSRA). It was depicted that with the increase in concentration

of phytochemicals, the percentage of inhibition of free radicals also increased. It was noticed that flavonoids and phenolics showed significant inhibition of free radicals. The antioxidant activity of these phytochemicals was higher than that of standard antioxidants. Alkaloids, glycosides, and saponins also showed comparable antioxidant activity; however, tannins and terpenoids show negligible antioxidant activity. The results of the study have similar findings as those previously reported [41, 42].

5. CONCLUSION

It can be concluded that the primary antibacterial components of *C. colocynthis* fruit extracts, which account for the majority of their antimicrobial action, are alkaloids. The flavonoids and phenolics in *C. colocynthis* fruit extracts have excellent potential as free radical scavengers. Thus, it can be said that the primary components of *C. colocynthis* for antioxidant activity are flavonoids and phenolics.

6. CONFLICT OF INTEREST

The authors declared no conflict of interest.

7. REFERENCES

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