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Effect on Antioxidant and Antibacterial Activity of Polyphenol Rich Fraction form Flowers of *Cassia occidentalis*

***P. Bama[1], S. Sankaranarayanan[2] and K. Prakash[3]**

[1]Professor, Department of Biochemistry, Sri Sairam Siddha Medical College and Research centre, Poonthadalam, West Tambaram, Chennai-600 048.

[2]Assistant Professor, Department of Medicinal Botany, Government Siddha Medical College, Arumbakkam, Chennai-600 106.

[3]Assistant Professor, Department of Botany, Arignar Anna Government Arts College, Villupuram-605 602.

Abstract

Traditionally, *Cassia occidentalis* flower has been extensively used to delicacy numerous infectious diseases. The aims of this examination were to determine the total phenol and flavonoid content of this plant using spectrophotometer; and assess the antioxidant and antibacterial possessions of the polyphenol rich fraction form flowers of *C. occidentalis*. The total flavonoid, and phenolic content assays showed that the polyphenol rich fraction had higher phenolic. The ABTS radical, Lipid peroxidation metal chelating, superoxide radical, nitric oxide scavenging and reducing power abilities and antibacterial activity were also evaluated. The flower polyphenol rich fraction revealed higher antioxidant properties of ABTS radical (72.31%), Lipid peroxidation (66.34%) metal chelating (78.32%), superoxide scavenging activity (71.56%) and Nitric oxide scavenging activity (81.45%). The antibacterial properties of the polyphenol rich fraction were analyzed by the disc diffusion method, and the flower extracts had higher antibacterial activities against the four bacterial strains used in the study. This study provides information on the synergistic antioxidant and antibacterial properties of phenolics derived from the flower parts of *C. occidentalis*.

Key words: *Cassia occidentalis;* polyphenol rich fraction; antibacterial; antioxidant

INTRODUCTION

Plant contained so many metabolites currently used as drugs and remain a vital role of food and pharmaceutical industry for convenience, reasonably low-cost cost and non-pollution in nature when related to modern chemicals (Shakya, 2016). Free radicals twisted in the human body respond with several biological material namely lipids, proteins and deoxyribonucleic acids resultant in the unevenness between oxidants and antioxidants. Even though human body is protected by natural antioxidant defense, there is continuously a request for antioxidants from natural sources. Phenolic compounds from therapeutic plants have tough antioxidant activity and may benefit to defend the cells in contrast to the oxidative damage triggered by free-radicals (Sajid *et al.,* 2012). They are well known as radical scavengers, metal chelators, reducing agents, hydrogen donors, and singlet oxygen quenchers (Ben Yakoub*et al.*, 2018). Antioxidants chemical substance from plant materials dismiss the exploit of free radicals in that way defensive the body from various diseases including age factor diseases.

Polyphenols are important chemical substance of plants and are commonly convoluted in protection in contradiction of ultraviolet radiation or violence by pathogens. Polyphenols may contribute in food industry to the bitterness, astringen, color, flavor, odor and oxidative stability. In the direction of the end of 20th century, epidemiological reports and related meta-analyses powerfully recommended that chronic ingesting of diets rich in vegetable polyphenols accessible roughly defense against growth of cancers, cardiovascular problem, and diabetes, neurodegenerative diseases and osteoporosis (Fernandez *et al.*, 1996). Polyphenols content plants are the subject of growing systematic attention since their probable helpful properties on human health. More than 8,000 polyphenol compounds have been isolated in countless plant species. Completely plant phenolic compounds stand up from a mutual midway, phenylalanine, or a near ancestor, shikimic acid. Mainly they occur in conjugated forms, with one or more sugar remains related to hydroxyl groups, though direct linkages of the sugar (polysaccharide or monosaccharide) to an aromatic carbon also exist.

Antibacterial fight is different as the struggle of bacteria to conduct with antibiotic drugs that was primarily initiate to be operative for the behavior of infection caused by that microorganism. This properties that antibiotics grow ineffective in conflict of resistant bacteria permitting impurities to persist in patients, pushing them at improved danger of worse clinical consequences and death. In detail, on regular, the humanity frequency for patients with contaminations produced

by non-resistant bacteria is fewer than partial of that of persons with an unaffected form of the similar infection (Bhatt and Negi, 2012). It has been systematically established that the unselective and unsuitable use of antibiotics has enhanced the appearance of drug-resistant strains. In addition, deprived hygienic conditions and infrequent food-handling inspire the additional spread of antimicrobial resistance. Since that antibacterial resistance is a multifactorial problematic, obsessed by many consistent factors, the World Health Organization proposes a successions of concentrated and corresponding movements (Brand-Williams *et al.*, 1995).

Cassia occidentalis is an annual herb. Leaves are arranged pinnate compound, leaflets are lanceolate, glossy leaf surface with characteristic odour and bitter taste; stem is hard and woody. Flowers are yellow in colour with 1 to 2 cm diameter arranged in axillary cyme and also forming terminal. Fruits are flat pods, 10 cm long with 10-15 seeds. Areolate seeds are pointed at end and blunt. The whole plant of *C. occidentalis* contain achrosin, aloe-emodin, emodin, anthraquinones, anthrones, apigenin, aurantiobtusin, campesterol. In traditional medicine, seed powder (half a tea spoon) recommended to cure fever although two table spoons of leaf juice mixed with honey cures cough. Leaf decoction half a cup used for intestinal gas and paste of leaf is applied for skin diseases. *C. occidentalis* extract also demonstrated that it prevents the carbon tetrachloride induced hepatotoxicity in rats. Finding on the statement they proposed that Himoliv increases the protective enzymes superoxide dismutase (SOD) and catalase in liver homogenate of rats. There is conversely lack of information on proportional evaluation of the antimicrobial and antioxidant properties of polyphenol rich fraction form flowers of *C. occidentalis*; hereafter this present study aimed to assess the antioxidant and antibacterial properties of the flower polyphenol extract of *C. occidentalis*.

MATERIALS AND METHODS

PLANT MATERIAL

*Cassia occidentalis*was obtained from Herbal garden of Hovernment Siddha Medical College, Arumbakkam, Chennai, Tamilnadu, India. A plant taxonomist authenticated the plant and samples were kept in the Medicinal Botany herbarium with voucher specimen numbers MB/GSMC-123/2021. The flowers were sufficiently air-dried in 5 days at the ambient room temperature, while the flower was cut into smaller pieces and air-dried in 7 days.

PHYTOCHEMICAL SCREENING

The aqueous extract of *Cassia occidentalis* flower were subjected to phytochemical screening to determine the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, glycosides, saponins and polyphenols using standard procedures (Aida et al., 2001; Hess et al., 1995).

TOTAL PHENOLIC CONTENT

The total phenolic content (TPC) of aqueous extract of *Cassia occidentalis* flower was determined using the method by Singleton, (1965). The aqueous extract (1 mL, 1 mg/mL) was mixed thoroughly with 1 mL of 50% Folin-Ciocalteu reagent and 1 mL of 2% Na_2CO_3, and centrifuged at 13400X *g* for 5 min. The absorbance of upper phase was measured using a spectrophotometer (ELICO (SL150) UV–Vis Spectrophotometer) at 750 nm after 30 min incubation at room temperature. Total phenolic content was expressed as a catechol equivalent.

ESTIMATION OF FLAVANOID

A 1ml aliquot of each aqueous extract of *Cassia occidentalis* was mixed thoroughly with 1ml of 2% aluminium chloride and 0.5 ml 0f 33% acetic acid followed by the addition of 90% methanol and the content is thoroughly stirred and allowed to stand for 30 minutes (Elfalleh et al., 2019). The absorbance was measured at 414 nm using a UV-Visible Spectrophotometer. Quercetin was used as a standard.

EXTRACTION OF POLYPHENOLS

Polyphenols were extracted from crushed flower of *Cassia occidentalis* (100 g), according to the method of Zhang et al. (2000). The fraction was completed twice at 20 °C in a shaking incubator. Methanol/acetone/water (3.5:3.5:3, v/v/v) containing 1 % formic acid were used extracting solvents were 100 mL at 30 min. The extract was then filtration through Whatman No.1 filter paper. The filtrates solution were evaporated under vacuum at 40 °C to remove methanol and acetone. Lipophilic colours materials were removed from the aqueous phase by two consecutive extractions in a separator funnel with a twofold volume of petroleum ether. The aqueous phase was finally collected and further extracted three times by ethyl acetate (ethyl acetate: aqueous phase = 1:1, *v/v*) in the separator funnel. The ethyl acetate phases were collected, evaporated and dried under vacuum at 35 °C to obtain polyphenol sample.

ABTS *(*2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) RADICAL SCAVENGING ASSAY

ABTS radical scavenging activity of polyphenol rich fraction form flowers of *C. occidentalis* flowerwas followed by Re et al. (1999). ABTS radical was newly prepared by addition 5 ml of 4.9 mM potassium persulfate solution to 5 ml of 14 mM ABTS solution and kept for 16 h in dark. This solution was diluted with distilled water to produce an absorbance of 0.70 at 734 nm and the same was used for the antioxidant activity. The final solution of standard group was made up to 1 ml with 950 µl of ABTS solution and 50 µl of Ascorbic acid. Correspondingly, in the experiment group, 1 ml reaction mixture encompassed 950 µl of ABTS solution and 50 µl of different concentration of each extracts. The reaction mixture was vortexed for 10 s and after 6 min, absorbance was recorded at 734 nm against distilled water by using a Deep Vision (1371) UV–Vis Spectrophotometer and compared with the control ABTS solution. Ascorbic acid was used as reference antioxidant compound.

ABTS Scavenging Effect (%) = $[(A_0 - A_1/A_0) \times 100]$ Where A_0 is the absorbance of the control reaction and A_1 is the absorbance of polyphenol rich fraction form flowers of *C. occidentalis*.

INHIBITION OF LIPID PEROXIDATION ACTIVITY

Lipid peroxidation induced by Fe^{2+}ascarbate system in egg yolk was assessed as thiobarbituric acid reacting substances (TBARS) by the method of Badmus et al. (2010). The experimental mixture contained 0.1 ml of egg yolk (25% w/v) in Tris-HCl buffer (20 mM, pH 7.0); KCl (30 mM); $FeSO_4$ $(NH_4)_2SO_4.7H_2O$ (0.06 mM); and different concentrations of flavonoid rich fraction from the flower of *Cassia alata* flowerin a final volume of 0.5 ml. The experimental mixture was incubated at 37°C for 1 h. After the incubation period, 0.4 ml was collected and treated with 0.2 ml sodium dodecyl sulphate (SDS) (1.1%); 1.5 ml thiobarbituric acid (TBA) (0.8%); and 1.5 ml acetic acid (20%, pH 3.5). The final volume was made up to 4.0 ml with distilled water and then kept in a water bath at 95 to 100 °C for 1 hour. After cooling, 1.0 ml of distilled water and 5.0 ml of n-butanol and pyridine mixture (15:1 v/v) were added to the reaction mixture, shaken vigorously and centrifuged at 4000 rpm for 10 min. The absorbance of butanol-pyridine layer was recorded at 532 nm in Deep Vision (1371) UV–Vis Spectrophotometer) to quantify TBARS. Inhibition of lipid peroxidation was determined by comparing the optical density (OD) of test sample with control. Ascorbic acid was used as standard.

Inhibition of lipid peroxidation (%) by the each extracts was calculated according to 1-(E/C) × 100, where C is the absorbance value of the fully oxidized control and E is absorbance of the test sample.

SUPEROXIDE RADICAL SCAVENGING ASSAY

This assay was based on the capacity of the polyphenol rich fraction form flowers of *C. occidentalis* to inhibit the photochemical reduction of Nitroblue tetrazolium (NBT) in the presence of the riboflavin-light-NBT system (Tripathi and Pandey Ekta, 1999; Tripathi and Sharma, 1999). Each 3 ml reaction solution contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 2 µM riboflavin, 100 µM Ethylene diamine tetra acetic acid (EDTA), NBT (75 µM) and different concentration of extracts. It was kept visible in fluorescent light and absorbance was taken after 6 min at 560 nm by using a Deep Vision (1371) UV–Vis Spectrophotometer. Identical tubes with reaction mixture were kept in the dark served as blanks. The percentage inhibition of superoxide radical activity was measured by comparing the absorbance of the control with test sample solution:

$$\% \text{ Super oxide radical scavenging capacity} = [(A_0-A_1)/A_0] \times 100$$

Where A_0 was the absorbance of control and A_1 was the absorbance of polyphenol rich fraction form flowers of *C. occidentalis*.

NITRIC OXIDE RADICAL SCAVENGING ACTIVITY

Nitric oxide scavenging ability of polyphenol rich fraction form flowers of *C. occidentalis* was measured according to the method described by Makhija et al. (2011). 0.1 ml of sodium nitroprusside (10 mM) in phosphate buffer (0.2 M, pH 7.8) was mixed with different concentration of extracts and incubated at room temperature for 150 min. After treated period, 0.2 ml of Griess reagent (1% Sulfanilamide, 2% Phosphoric acid and 0.1% N-(1-Naphthyl) ethylene diamine dihydrochloride) was added. The absorbance of the experimental sample was read at 546 nm against blank. All readings were taken in triplicate and ascorbic acid was used as standard. The percentage of inhibition was calculated by following equation:

$$\% \text{ Nitric oxide radical scavenging capacity} = [(A_0-A_1)/A_0] \times 100$$

Where A_0 was the absorbance of control and A_1 was the absorbance of polyphenol rich fraction form flowers of *C. occidentalis*.

METAL CHELATING ACTIVITY

Metal chelating capacity of polyphenol rich fraction form flowers of *C. occidentalis* was measured according to Dinis et al., (1994). 1 ml of different concentrations of flavonoid rich fraction was added to 0.05 ml of 2 mM ferric chloride solution. The reaction was initiated by the addition of 0.2 ml of 5 mM Ferrozine and the mixture was shaken vigorously. After 10 min, the absorbance was measured at 562 nm against blank. All readings were taken in triplicate and ascorbic acid was used as standard. The % inhibition of ferrozoine-Fe2+complex was calculated by following equation.

$$\% \text{ Inhibition of ferrozoine-Fe2+complex} = [(A_0-A_1)/A_0] \times 100$$

Where A_0 was the absorbance of control and A_1 was the absorbance of polyphenol rich fraction form flowers of *C. occidentalis*.

CULTURE COLLECTION AND MAINTENANCE

The bacterial strains of *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli* and *Pseudomonas aeruginosa.* These standard strains were obtained from Microbial Type Culture Collection and gene bank (MTCC); Institute of Microbial Technology, Chandigarh, India. The stock culture was maintained on Mueller Hinton agar medium at 4 ◦C.

ANTIBACTERIAL ACTIVITY OF FLAVONOID RICH FRACTION OF *CASSIA ALATA* FLOWER

The antibacterial activities of the polyphenol rich fraction were assayed using the disc diffusion method (Drago *et al.*, 1999). Bacteria were grown overnight on Mueller Hinton agar plates, five colonies were suspended in 5 ml of sterile saline (0.9%) and the bacterial population in the suspension was adjusted to ~$3x10^8$ CFU/ml. A sterile cotton swab was dipped into the suspension and the swab rotated several times with firm pressure on the inside wall of the tube to remove the excess fluid. The swab was used to inoculate the dried surface of MH agar plate by streaking four times over the surface of the agar, rotating the plate approximately by 90º to ensure an even distribution of the inoculums. The medium was allowed to dry for about 3 min before adding a sterile disc of 6 mm diameter. Each disc was placed firmly on to the agar to provide uniform contact with the bacteria. Bioactive compound (50 µg) was weighed and dissolved in 1 ml of 7% ethyl acetate. The different concentration of polyphenol rich fraction form flowers of *C. occidentalis* flower was introduced on to each disc and the control disc received only 7% ethanol. The plates were incubated at 37°C for 24 h and the inhibition zone was measured and calculated.

The experiments were carried out in duplicate three times. The results (mean value, *n*=3) were recorded by measuring the zones of growth inhibition surrounding the discs.

MINIMUM INHIBITORY CONCENTRATIONS (MICS)

The minimum inhibitory concentrations of the isolated compounds were determined by dilution method (Brantner and Grein, 1994). The strains were grown in Mueller Hinton broth to exponential phase with an *A*560 of 0.8, representing 3.2×10^8 CFU/ml. Different dilutions of the polyphenol rich fraction form flowers of *C. occidentalis* were prepared to give solutions of 25, 50, 75, and 100 µg/ml. 0.5 ml of each concentration was added into separate test tubes containing 4ml of MH broth inoculated with 0.5 ml bacterial suspension at a final concentration of 10^6 CFU/ml. Each MIC was determined from five independent experiments performed in duplicate. The tubes containing 4.5 ml of bacterial inoculates and 0.5 ml of 7% ethyl acetate used as bacterial control, 4.5 ml of uninoculated MH broth and 0.5 ml PBS served as a blank. The tubes were incubated at 37 ◦C for 18 h; inhibition of bacterial growth was determined by measuring the absorbance at 560 nm.

STATISTICAL ANALYSIS

The outcomes are shown as mean ± S.E.M. (n = 6). Statistical significance was determined by one-way analysis of variance with $p < 0.01$ and $p < 0.05$ considered significant followed by Dunnett Multiple Comparisons Test.

RESULT AND DISCUSION

PHYTOCHEMICAL SCREENING

The phytochemical screening of aqueous flower extract of *C. occidentalis* studied presently showed the presence of alkaloids, flavonoids, polyphenol, terpenoids, and absence of glycosides and tannin (Table -1). Plant secondary metabolites such as tannins, saponins, flavonoids, alkaloids and other plant metabolites compounds are serve as protection against predation by many microorganism include insects and other herbivores (Bonjar et al., 2004). This can partially explain the demonstration of antimicrobial and antioxidant activities by the flower of *C. occidentalis*.

Table-1. Phytochemical screening of aqueous flower extract of *C. occidentalis*

Sl. No.	Phytochemical Constituents	Observation	Aqueous flower extract of *C. occidentalis*
1	**Alkaloids** -Dragendorff's Test -Mayers test	Orange / red precipitate Yellow or white precipitate	+ +
2.	**Flavonoids** -Alkalai Reagent -Lead acetate test	Intense yellow colour Precipitate formed	+ +
3.	**Glycosides** Keller-Killiani test	Reddish brown colour ring formed	-
4.	**Tannin** -$FeCl_3$ test	Blue black coloration	-
5.	**Saponins** -Frothing test	Foam	+
6.	**Terpenoids** *-Salkowski test*	Dark reddish brown color in interface	-
7.	**Polyphenols** -Ferrozine test	Raddish blue	+
8.	**Anthocyanin test Ammonia**	Ammonia layer yellow in color	+

+ indicate positive result; -- Indicate negative result

TOTAL PHENOLIC AND FLAVONOID CONTENT

In this study, the initial experiments exposed that flower aqueous extraction of *C. occidentalis* flower 60 °C for 60 min since it afforded a maximum yield of phenolics. The total phenolic content of the aqueous fresh extract, calculated from the calibration curve (R^2 = 0.998), was 53.64 ±2.45 gallic acid equivalents/g, and the total flavonoid content (R^2 = 0.999) was 41.23±1.89 rutin equivalents/g (Table-2).

Table-2. Yield and phenolic and flavonoid content flavonoid rich fraction of *Cassia alata* flower

Sample	Yield of extract (g/100 g of defatted Content)	Total phenolic content (mg catechin equivalents per gram extract)	Total flavonoid content (mg catechin equivalents per gram)
Aqueous extraction of *C. occidentalis*	71.23±0.56[a]	53.64±2.45[b]	41.23±1.89

[a]Data are expressed as mean ± standard deviation (n = 3) on a fresh weight basis.

[b]Means in each column sharing the same letter are not significantly (P = 0.05) different from other.

ABTS RADICAL ASSAY

The antiradical activity of polyphenol rich fraction form flowers of *C. occidentalis*, as the demonstrative of nutritional food source, were evaluated *in vitro* by ABTS assay, as well as by assessment of possible to discoloration of ABTS. Substantial changes were witnessed for the polyphenol rich fraction, as well as between the assays employed. In table-3 the outcomes of antioxidant activity gained for tested samples, as well as Vitamin-C used as standard are presented. It can be evidently understood that polyphenol rich fraction exhibited prominent antioxidant activity, expressively higher than Vitamin-C. Nevertheless, in current experimental presented that these activities were mainly due to presence of polyphenol compounds. As stated by Rossetto et al. (2005), the presence of phenolics advises to Cassia sp. a remarkably high peroxyl radical scavenging properties in terms of both capacity and competence, mainly in their initial stage of growth.

Table-3. Free radical-scavenging ability using ABTS assay of polyphenol rich fraction of *C. occidentalis*

Different concentration of extract	ABTS radicalactivity	
	Polyphenol rich fraction form flowers of *C. occidentalis*	Standard Vitamin-C
25 µl/ml	19.63±2.41	15.63±1.78
50 µl/ml	36.21±0.58	31.24±0.86
75 µl/ml	54.21±1.36	47.32±0.56
100 µl/ml	72.31±2.45	64.53±2.18
EC_{50} value	56.32	66.23

[a]Results are expressed as percentage inhibit of ABTS ability with respect to control. Each value represents the mean+SD of three experiments

INHIBITION OF LIPID PEROXIDATION ACTIVITY

Inhibition of lipid peroxidation experimental was used as substrate egg yolk for free radical facilitated lipid peroxidation, which is a non-enzymatic method. Polyphenol rich fraction of *C. occidentalis* inhibited the lipid peroxidation brought by ferrous sulfate in egg yolk homogenates. Determined inhibition was documented in polyphenol rich fraction of *C. occidentalis* 66.34% with EC_{50} value 62.35 µl/ml and lowermost inhibition percentage ascorbic acid 61.23% with EC_{50} 71.23 (Table-4). As it is recognized that lipid peroxidation is the remaining outcome of any free radical attack on membrane and other lipid components present in the system, the lipid peroxidation may be enzymatic (Fe/NADPH) or non-enzymatic (Fe/ascorbic acid). Normally, the mechanism of polyphenol compounds for neutralizing lipid free radicals and stopping breakdown of hydro peroxides into free radicals (Gulçin, 2012).

Table-4. Inhibition of lipid peroxidation activity of polyphenol rich fraction of *C. occidentalis*

Different concentration of extract	Inhibition percentage of Lipid peroxidation	
	Polyphenol rich fraction of *C. occidentalis*	Standard Vitamin-C
25 µl/ml	17.32±2.34	14.56±1.43
50 µl/ml	31.25±1.47	27.36±0.87
75 µl/ml	51.49±2.36	46.32±2.13
100 µl/ml	66.34±1.56	61.23±2.58
EC_{50} value	62.35	71.23

[a] Results are expressed as percentage inhibit of lipid peroxidation with respect to control. Each value represents the mean+SD of three experiments.

SUPEROXIDE SCAVENGING ACTIVITY

Polyphenol rich fraction of *C. occidentalis* displayed authoritative scavenging activity for superoxide radicals in a concentration dependent development than positive control. Polyphenol rich fraction of *C. occidentalis* exhibited maximum radical activity in the percentage of 71.56% with EC_{50} value 59.32 µl/ml when related to positive control 63.21% with EC_{50} Value 65.21 µl/ml (Table-5). One of the typical process to produce Superoxide radicals is concluded photochemical decrease of nitro blue tetrazolium (NBT) in the presence of a riboflavin-light-NBT system. These superoxide radicals are enormously toxic and may be twisted either through xanthine activity or through mitochondrial reaction. Superoxide radical is recognized to be an identical damaging species to cellular components as an ancestor of additional oversensitive specie. The superoxide radical is identified to be produced *in vivo* and can outcome in the development of hydrogen peroxide *via* dismutation reaction. The result obviously specifies that the polyphenol compound has an evident outcome as superoxide radical scavenging properties (Sharma and Gupta, 2008).

Different concentration of extract	Percentage of Superoxide scavenging activity	
	Polyphenol rich fraction of *C. occidentalis*	Standard Vitamin-C
25 µl/ml	23.56±1.49	16.32±0.87
50 µl/ml	39.64±0.23	33.64±1.56
75 µl/ml	56.34±2.15	52.78±2.63
100 µl/ml	71.56±0.86	63.21±2.45
EC_{50} value	59.32	65.21

Table-5. Superoxide scavenging activity of polyphenol rich fraction of *C. occidentalis*

[a] Results are expressed as percentage of Superoxide scavenging activity with respect to control. Each value represents the mean+SD of three

METAL CHELATING ACTIVITY

The metal chelating properties was conducted by polyphenol rich fraction of *C. occidentalis* displayed as per Table-6. Polyphenol rich fraction were assessed for their capacity to contest with ferrozine for ferrous iron in the solution. In this estimation, the polyphenol rich fraction delayed the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and are gifted of arresting ferrous iron before ferrozine. The polyphenol rich fraction condensed the greenish blue color complex instantly and showed the highest chelating activity 78.32% With EC_{50} Value 51.23 µl/ml than positive control Vitamin-C 72.32% with EC_{50} value 57.42 µl/ml.Iron toxicity is related with an enlarged danger of free radical damage and cancer. Chelation treatment may feasibly decrease iron connected free radical damage and increase the general survival in cardiovascular diseases (Halliwell, 1991).

Table-6. Metal chelating activity of polyphenol rich fraction from. *C. occidentalis* flower

Different concentration of extract	Percentage of Metal chelating activity	
	Polyphenol rich fraction from *C.occidentalis*	Standard Vitamin-C
25 µl/ml	21.53±2.89	16.32±3.6
50 µl/ml	35.21±2.47	27.58±0.58
75 µl/ml	54.32±0.89	48.32±2.89
100 µl/ml	78.32±2.56	72.34±1.46
EC_{50} value	51.23	57.42

[a]Results are expressed as percentage of Metal chelating activity with respect to control. Each value represents the mean+SD of three experiments.

NITRIC OXIDE RADICAL SCAVENGING

Polyphenol rich fraction of *C. occidentalis* indicated a strong nitric oxide scavenging ability which was equivalent to the standards ascorbic acid. The EC_{50} value (47.23) of polyphenol rich fraction of *C. occidentalis* was less than ascorbic acid (52.34). Percentage of nitric oxide radical scavenging activity polyphenol rich fraction of *C. occidentalis* and standards were presented in Table-7. In the present outcome, nitrite was formed by incubation of sodium nitroprusside in standard phosphate saline buffer at 25°C was reduced by polyphenol rich fraction. Imperative scavenging activity may be due to the antioxidant property of polyphenol, compounds present in *C. occidentalis*flower, which contest with oxygen to respond with nitric oxide, prominent to less production of nitric oxide. Blando *et al.* (2019) evaluated the antioxidant activity of polyphenolic extracts from cladodes of *Opuntia ficus-indica*. In particular, O. ficus-indica cladodes extracts exhibited in vitro strong free radical scavenging activity and increased antioxidant activity in human erythrocytes

Table-7. Nitric oxide radical scavenging assay of polyphenol rich fraction of *C. occidentalis*

Different concentration of extract	Percentage of Nitric oxide radical scavenging activity	
	Polyphenol rich fraction of *C. occidentalis*	Standard Vitamin-C
25 µl/ml	24.58±0.56	17.32±1.87
50 µl/ml	41.35±1.78	33.21±1.56
75 µl/ml	62.31±2.89	58.34±2.68
100 µl/ml	81.45±1.58	75.31±1.47
EC_{50} value	47.23	52.34

[a] Results are expressed as percentage of Nitric oxide radical activity with respect to control. Each value represents the mean+SD of three experiments.

EFFECT OF FLAVONOID POLYPHENOL RICH FRACTION OF *C. OCCIDENTALIS* ON THE GROWTH OF PATHOGENIC BACTERIA BY DISC DIFFUSION METHOD

Antibacterial activity of polyphenol rich fraction of *C. occidentalis*tested against *Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa*and *Escherichia coli* were assessed as inhibition zones in the agar plates (Table-8). In this experimental all the bacteria were found to be sensitive to the polyphenol rich fraction. Additionally, the zone of inhibition reconsideration that the polyphenol rich fraction influenced antibacterial activity in proportion to concentration gradient ranges 25-100 µl/ml against the tested bacteria. Amongst the bacteria considered, *Staphylococcus aureus* and *Escherichia coli* was identified to be highly susceptible followed by *Pseudomonas aeruginosa* and *Enterococcus faecalis*. This may confirm the antibacterial property of polyphenol rich fraction from the flower of *C. occidentalis*. Polyphenols metabolites such as phenolic and flavonoids, are significant antibacterial activity (Machado *et al.*, 2002). The antimicrobial activity of polyphenols are due to their capability to composite with extracellular and soluble protein and to complex with bacterial cell wall although polyphenol may be related to their ability to deactivate microbial adhesions, enzymes and cell envelop proteins (Cowan, 1999).

Table-8. The antibacterial activity of the polyphenol rich fraction from the flower of *C. occidentalis*by disc diffusion method

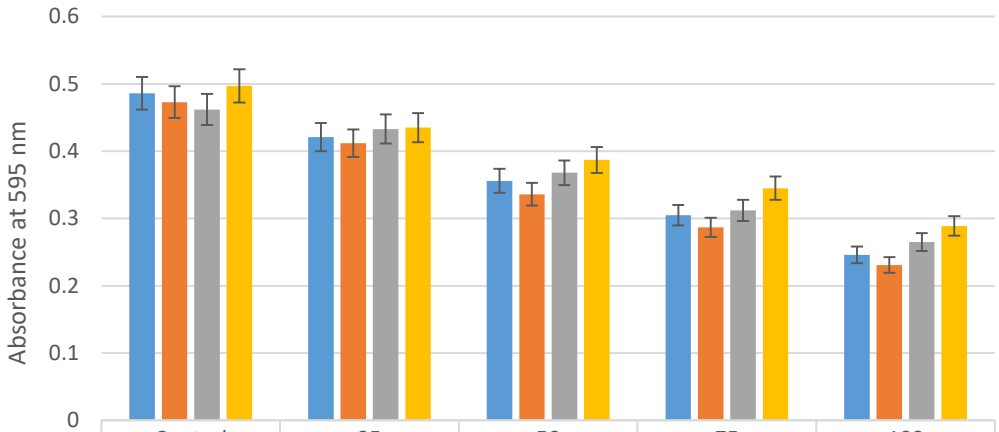
Pathogenic organism	Different concentrations Crude extract (µl/ml)			
	25 µl/ml	50 µl/ml	75 µl/ml	100 µl/ ml
Staphylococcus aureus	9.5±0.2	11.8±1.2	14.5±0.4	16.3±1.3
Pseudomonas aeruginosa	7.4±2.5	9.8±1.5	12.1±1.3	14.6±0.5
Escherichia coli	8.7±1.3	10.6±0.8	13.7±1.6	15.7±1.8
Enterococcus faecalis	7.1±0.9	9.5±2.4	11.6±1.4	13.4±2.3

*The inhibitory Zone size measured included the 6.0 mm size of the well by means of caliper. All the assays were duplicated, and the mean values were recorded.

MINIMUM INHIBITORY CONCENTRATION

In the complete sequences, the MIC of polyphenol rich fraction of *C. occidentalis* flower ranged between 25 to 100 µg/ml against gram positive bacteria and gram negative bacteria, (*Staphylococcus aureus, Enterococcus faecalis, Escherichia coli* and *Pseudomonas aeruginosa*) respectively. The Minimum inhibitory absorption value of polyphenol rich fraction of *C. occidentalis* increases with increase in concentration.*S. aureus* exhibited maximum inhibition when compared to the other pathogenic bacteria at 100 µl/ml concentration.*Enterococcus faecalis, Escherichia coli* appearances reasonable range of inhibition activity.*P. aeruginosa* display slighter activity. In comparison with gram positive bacteria and gram negative bacteria, the MIC of polyphenol rich fraction of *C. occidentalis*displayed highest inhibition in gram negative bacteria and among the gram positive bactertia *S. aureus*showed maximum inhibition (Graph-1).

Graph-1. The antibacterial activity of the polyphenol rich fraction from the flower of *C. occidentalis* by MIC method



	Control	25	50	75	100
Staphylococcus aureus	0.486	0.421	0.356	0.305	0.246
Escherichia coli	0.473	0.412	0.336	0.287	0.231
Enterococcus faecalis	0.462	0.433	0.368	0.312	0.265
Pseudomonas aeruginosa	0.497	0.435	0.387	0.345	0.289

Staphylococcus aureus Escherichia coli Enterococcus faecalis Pseudomonas aeruginosa

CONCLUSION

The polyphenol rich fraction of *C. occidentalis*of the flowers were establish to have momentous antibacterial and antioxidant activities. The flowers contained the highest total phenolic and flavonoid amount, which clarifies polyphenol rich fraction of *C. occidentalis*had superior antibacterial and antioxidant effects. This experimental confirmations that there are synergistic antimicrobial and antioxidant abilities that are derived from polyphenol compounds in flowers of *C. occidentalis*.

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