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Antimicrobial, Antioxidant and Cytotoxic Potential of *Caesalpinia pulcherrima* Flower

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Abstract: Most of the world's populations residing in developing countries depend on alternative medicine and use of plant ingredients. *Caesalpinia pulcherrima* (Fabaceae) is used in folk medicine for the treatment of various diseases including asthma, bronchitis, cholera, diarrhea, dysentery and malarial infection. The present study was undertaken to determine antimicrobial, antioxidant and cytotoxic activities of flower extracts of this Ayurvedic plant. Antimicrobial activity was tested against *Staphylococcus aureus* (G+ve bacteria), *Pseudomonas aeruginosa* (G-ve bacteria), *Candida albicans* (yeast) and *Aspergillus Niger* (Fungi) by Disc agar plate method. Antioxidant activity was determined by DPPH free radical scavenging assay, Ferric reducing assay and total antioxidant capacity determination. Cytotoxic activity was evaluated via using preliminary brine shrimp lethality test and toward liver cancer cell line; HepG2. Among the methanol extracts and the fractions, butanol fraction showed the highest phenolic content (490.52 mg gallic acid/g) and the antioxidant capacities of the 90% methanol extract were 444.4, 0.679, 19.11µmol/g in TAC, RPAA and DPPH assays, respectively. This study verified that the extracts and butanol fraction from *C. pulcherrima* have strong antioxidant activity that was correlated with their high level of phenolic content. Furthermore, the same extract showed appreciable cytotoxic activity via HEPG2 assay, the IC_{50} 48.6 µg/mL. A marked antimicrobial and antioxidant activity of flower extracts was observed which may be attributed to the presence of phenolic compounds and other phytochemicals. It is concluded that C. *pulcherrrima* can be used to control infectious diseases and oxidative damage.

Key words: *Caesalpinia pulcherrima* · Antimicrobial · Antioxidant · Cytotoxic · Total Phenolics

INTRODUCTION

It is estimated that about 85% of people living in developing countries depend on traditional medicine [1]. These are used not just in rural areas of developing countries, but also in developed countries where modern medicines are predominantly used [2]. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases. The medicinal value of plants lies in certain chemical substances that produce a definite physiological action on the human body. The most important among these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds [3].

Free radicals are considered as the products of normal metabolic processes in the human body, but when produced in excess, they cause damage to biomolecules [4]. Oxygen radicals generated during reduction of oxygen can attack DNA bases or deoxyribose residues to produce damaged bases or strand breaks [5]. Such biomolecular damage by free radicals leads to many pathological diseases such as cancer, inflammation and atherosclerosis [6]. On the other hand, it is known that many natural products contain important combinations of ingredients that may to some extent help to modulate the effects produced by oxidation substrates in biological systems [7].

Infectious diseases are caused due to a complex interaction between the pathogen, host and the environment. The discovery of antibiotics and their subsequent use had eradicated the infections that once challenged mankind. However, therapy using antibiotics is going through a crisis due to development of resistance by pathogens. *Staphylococcus aureus* is one of the most

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important pathogens that have become resistant to almost all known antibiotics. Other examples for antibiotic resistant bacteria are vancomycin resistant enterococci, multidrug resistant tuberculosis and others. Moreover, these pathogens have the ability to transmit the resistance gene and thereby create a serious issue in the field of medicine [8]. Plants have been used long before the discovery of antibiotics as remedies for a number of human diseases. They contain a great array of secondary metabolites having therapeutic value. Traditional healers often referred as herbal healers, from various parts of the world use plants as anti-infective agents. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [9].

Caesalpinia pulcherrima (Fabaceae), popularly known as peacock flower or "Barbados pride" in English and as "Mayil kondrai" in Tamil, it is a large tree that is found mainly in the tropical and subtropical zones. In folk medicine, *Caesalpinia pulcherrima* has been used for the treatment of various diseases including asthma, bronchitis, cholera, diarrhea, dysentery and malarial infection [10]. The flowers of *C. pulcherrima* have been reported to possess antiviral activity [11]. Therefore, the current study was undertaken to evaluate the antioxidant, cytotoxic and antimicrobial activities of 90% MeOH extract of *C. pulcherrima* as well as its derived fractions.

MATERIALS AND METHODS

Plant Material: The flowers of *Caesalpinia pulcherrima* (Fabaceae) were collected from Zoological Garden, Giza, Egypt in August-October, 2012. Authentication of the plant was established by Eng. Teresa Labib, General Manager and head of plant Taxonomy in El-Orman Botanical Garden, Giza, Egypt. Voucher specimen was deposited at Laboratory of Medicinal Chemistry, Theodor Bilharz Research Institute. The fresh flowers were washed with clean water and completely dried in shade place at room temperature and then powdered by electric mill.

Extraction and Fractionation: Extraction was done at room temperature by simple extraction method using different solvents namely, 100% Methanol, 90% Methanol, 85% Methanol and 70% Methanol. Dried powdered leaves (15 g) were mixed separately with 100 ml of each solvent in 500 ml conical flasks. The flasks were sealed tightly and kept for 24 hrs. The supernatant was filtered using Whatman filter paper No.1 and evaporated using a rotary evaporator (Rotatory evaporator. Buchi, Switzerland) to obtain the crude dried extract. Large scale extraction was carried out via taking the plant powder (500 g), was soaked in (2L) of 90% methanol for one week at room temperature. The 90% methanol extract was defatted with diethyl ether and then fractionated by using different organic solvents; petroleum ether, methylene chloride, EtOAc and n-BuOH. Each fraction was filtered and then concentrated. The yield of each fraction was determined and kept in dark for analysis.

Phytochemical Screening: Phytochemical screening for the secondary metabolites (Alkaloids, tannins, sterols, saponins, glycosides, sterols/Terpenes, sugars, flavonoids and phenols) was carried out by the reported methods Khandelwal [12], Mabry *et al.* [13] and Harborne [14].

Determination of Total Phenolic Content: The total phenolic content of each extract was determined using Folin- Ciocalteu's reagent [15]. Gallic acid was used as standard. In this method, the reaction mixture was composed of (100 µL) of plant extract (100 µg/mL) and 500 µL of the Folin-Ciocalteu's reagent and 1.5 mL of sodium carbonate (20%). The mixture was shaken and made up to 10 mL using distilled water. The mixture was allowed to stand for 2 h. Then the absorbance was measured at 765 nm using spectrophotometer (UV-VS spectrophotometer, Milton Roy 601, Co, USA). All determinations were carried out in triplicate. The total phenolic content was expressed as mg Gallic acid equivalent (GAE) per g extract [16].

Determination of Total Flavonoids Content: The content of flavonoids of each extract was determined according to the reported procedures by Lamaison and Carnat [17] using rutin as a standard. Briefly, 100 µL of plant extract in methanol (100 µg/mL) was mixed with 100 µL of aluminium trichloride ($AlCl_3$) in methanol (20 mg/mL) and then diluted with methanol to 500 µL. The absorption at 415 nm was read after 40 min against the blank. The blank consists of all reagents and solvent without $AlCl_3$. All determinations were carried out in triplicate. The total flavonoid in plant extracts was determined as mg rutin equivalents (RE)/g extract [18].

Antimicrobial Assay: Disc agar plate method was done to evaluate the antimicrobial activity of different methanol extracts and their derived sub-fractions from the selected plants. The antimicrobial activities of 0.5-cm-diameter filter paper disc saturated with about 1mg sample were tested against four different microbial strains, i.e.,

Staphylococcus aureus (G+þve bacteria), *Pseudomonas aeruginosa* (G-ve bacteria), *Candida albicans* (yeast) and *Aspergillus niger* (fungi). Both bacterial and yeast test microbes were grown on nutrient agar (DSNZ 1) medium (g/l): beef extract (3), peptone (10) and agar (20). Whereas fungal test microbe was grown on Szapek-Dox (DSMZ130) medium (g/l): sucrose (30), $NaNO_3$ (3), $MgSO_4.7H_2O$ (0.5), KCl (0.5), $FeSO_4.7H_2O$ (0.001), K_2HPO_4 (1) and agar (20). The culture of each microorganism was diluted by sterile distilled water to 10^7 to 10^8 CFU/ml to be used as inoculum.1ml of the previous inoculum was used to inoculate 1l of agar medium (Just before solidification) then poured in Petri-dishes (10cm diameter containing 25ml). Discs (5 mm diameter) were placed on the surface of the agar plates previously inoculated with the test microbe and incubated for 24 h for bacteria and yeast but for 48 h for fungus at 37 and 30°C, respectively [19, 20].

Antioxidant Assay

Determination of Total Antioxidant Activity (TAA): Total antioxidant activity (TAA) of each sequential extract was determined according to the method of Prieto *et al*. [21]. Briefly 0.3ml dilute concentration of leaf and flower extracts was mixed with 3ml of reagent solution (0.6M Sulfuric acid, 28mM Sodium phosphate and 4mM Ammonium molybdate) in labeled tubes. The tubes were capped and incubated in boiling water bath at 95°C for 90 min. The tubes were cooled and the absorbance of the solution was measured at 695nm using a spectrophotometer. In case of blank 0.3ml of methanol was used in place of extracts. Ascorbic acid was used as reference standard and the antioxidant capacity of extracts was expressed as µg ascorbic acid equivalents (AAE)/mg of extract [22].

Scavenging Effect on 1, 1'-diphenyl-2-picrylhydrazyl Radical (DPPH): The different concentrations of each of the extracts were prepared in methanol and were added to 3ml of 0.1mM methanolic solution of DPPH. Control was prepared containing the same solvents and reagents without any extract. The tubes were shaken vigorously and allowed to stand for 20 min at room temperature in the dark. Changes in absorbance of samples were measured at 517 nm. A control reading was obtained using methanol instead of the extract. Ascorbic acid served as the standard. Free radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula,

$$\% \text{ inhibition} = ((A_0 - A_t) / A_0 * 100$$

where, A_0 is the absorbance of the control, A_t is the absorbance of test samples. All the tests were performed in triplicates and the results are reported as SC_{50} which is the amount of antioxidant necessary to decrease the initial $DPPH^{\bullet}$ concentration by 50%. The lower SC_{50} value corresponds to a higher scavenging activity (Higher antioxidant activity) of plant extract [23-24].

Reducing Power Antioxidant Assay (RPAA): For this 2.5 ml of each of the extracts was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide (10 mg/ml). The mixture was incubated at 50°C for 20 min, then rapidly cooled, mixed with 2.5 ml of 10% trichloroacetic acid and centrifuged at 6500 rpm for 10 min. An aliquot (2.5 ml) of the supernatant was diluted with distilled water (2.5 ml) and then ferric chloride (0.5 ml, 0.1%) was added and allowed to stand for 10 min. The absorbance was read spectrophotometrically at 700 nm. Ascorbic acid used as standard. Three replicates were made for each test sample. The percentage of reducing power was calculated by using the formula:

$$\text{Reducing power (\%)} = A_{control} - A_{sample} / A_{control}$$

where, $A_{control}$ was the absorbance of solution without extract and A_{sample} was the absorbance with different dilutions of extract, Ascorbic acid was used as a standard [25].

Cytotoxic Activity

Toxicity Testing Using the Brine Shrimp (Artemia Saline): A simple animal that has been used to monitor lethality is the brine shrimp, *Artimia salina* Leach [26]. *Artemia salina* L (*Artemiidae*), commonly known as the brine shrimp, is a small crustacean used to determine the toxicity of a wide variety of products. This assay is considered as one of the most useful tools for a preliminary assessment of toxicity of plant extracts. A large Petri dish is divided by a glass that does not quite reach to the bottom of the dish and is filled with the sterilized seawater. Cysts (About 100 to 200 mg) are placed in one compartment and a 60 watt lamp is position to provide direct light and warmth (About 25° C) through the embryogenesis. Free swimming nauplii appear after 16 h and most of the eggs should have hatched into free-swimming form by 24 h. The free-swimming nauplii should be ready for collection some 36 to 48 h after sowing. These may be attracted to the other compartment, which is free from emerging embryos, eggs and egg debris, by a torch light. The nauplii can be collected using

a Pasteur pipette with a nozzle of at least 1 mm diameter [27]. The assay is begun 36 to 48 h after sowing of the cysts (i.e., with larvae that are 20 to 32 h old). Multi welled culture plates can be used the bioassay, although any clear glass container with flat bottom (For example, small beakers or glass vials) will do. Ten nauplii are collected, using a Pasteur pipette, from the hatching dish and transferred to a well, using the minimum amount of seawater. Two milliliters of the test solution are added and the time is noted. This is repeated for two additional wells, thereby requiring 30 nauplii for each concentration of test sample. A parallel series of tests with the standard potassium dichromate solution (800 ppm, 600 ppm, 400 ppm) and the blank control are always conducted. To determine the acute LC_{50}, the acute number of dead nauplii is counted in every well after 6 h. counting for the chronic LC_{50} begins 24 h after initiation of the tests. Nauplii are considered dead if they are lying immobile at the bottom of the well. A hand lens is useful to check for inactivity of the appendages (The antennae and the mandibles). Alternatively, the whole tray can be put on overhead projector and the silhouette, focused on the bottom of the wells, is projected upon a screen. It is then easy to count the dead animals. Live nauplii should then be killed by addition of a few drops of formaldehyde solution and the total dead counted to confirm the number of animals in each well Sam [26]. The Reed-Muench method assumes that an animal that survived a given dose would also have survived any lower dose and conversely, that an animal that died with a certain dose would have also died at any other higher dose. Thus, the information from any one group can be added to that of the other groups in the range of dose [28-29].

Liver Carcinoma Cell Line (HepG2): Potential cytotoxicity of Butanol extract was tested using method of Skehan *et al*. [30], using cell line HEPG2. Cells were plated in 96-multiwell plate (104cells/well) for 24 h before treatment with the extract to allow attachment of cell to the wall of the plate. Different concentrations of the butanol extract (0, 1, 2.5, 5 and 10 µg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the extract for 48 h at 37 °C and atmosphere of 5 % CO2. After 48 h, cells were fixed, washed and stained with sulforhodamine B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration is platted to get the survival curve of each tumor cell line after the specified Extract [30]. The IC_{50} value was defined as the concentration of plant extract necessary to inhibit the growth to 50% of the control.

RESULTS AND DISCUSSION

Phytochemical Screening: Identification of the major chemical constituents of methanol extract and its derived fractions was carried out using the conventional standard procedures [3, 31-34], to determine presence or absence of the different phytoconstituents such as; alkaloids (Mayer's and Draggendorff's tests), flavonoids (Shinoda test, Aluminum chloride and Potassium hydroxide tests), steroids and terpenoids (Salkowski and Libarman-Burchard's tests), tannins (Ferric chloride and Gelatin tests), saponins (Frothing and Hemolytic tests), anthraquinones (Borntrager's test), carbohydrates (Molisch's and Barfoed's tests) and coumarins (Sodium hydroxide test). The results were evaluated by visual inspection as change in color or precipitation, showed the presence of certain secondary metabolites as glycosides, flavonoids, phenolics and tannins [3, 31-34].

Total Flavonoids and Total Phenolic Contents: The content of total phenolics in the flower extracts was estimated by FCR method. Total phenolic content, as estimated in terms of mg GAE/g extract, was high in butanol extract (490.52) followed by 90% methanol extract (280.85), ethyl acetate extract (242.98) and others. Diethyl ether extract contained lowest phenolic content. From analysis, it was found that all extracts of *Caesalpinia pulcherrima*, butanol and all ratios of methanol extracts are rich in flavonoids content but diethyl ether, petroleum ether and methylene chloride extracts have low content of flavonoids (Table 1). The previous studies revealed that the phenolic contents are positively correlated to the antioxidant activities; furthermore the antioxidant activities of phenolics are probably due to their redox features, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers [35].

Antimicrobial Activity: Result of inhibitory efficacy of flower extracts against four different microbial strains, i.e., *Staphylococcus aureus* (G+þve bacteria), *Pseudomonas aeruginosa* (G-ve bacteria), *Candida albicans* (Yeast) and *Aspergillus niger* (Fungi) are shown in (Table 2). For *Staphylococcus aureus* highest inhibitory efficacy was shown by 70% methanol extract of *C. pulcherrima* and for *Pseudomonas aeruginosa* the highest inhibition is 100% methanol extract. Inhibition of *Candida albicans*

Table 1: Total phenolic content (TPC) and total flavonoid content (TFC) of the different extracts of *Caesalpinia pulcherrima*

Sample	TFC (mg RE / g ext.)[a]	TPC (mg GAE / g ext.)[b]
100% MeOH	72.19 ± 2.16	169.10 ± 1.46
90% MeOH	94.27 ± 2.04	280.85 ± 2.89
85% MeOH	55.40 ± 1.01	162.11 ± 0.83
70% MeOH	31.78 ± 3.31	96.04 ± 3.12
Diethyl ether	5.19 ± 2.13	66.43 ± .00
Petroleum ether	16.18 ± 1.60	109.16 ± 2.54
Methylene chloride	21.23 ± 1.19	185.60 ± 3.08
Ethyl acetate	55.47 ± 3.29	242.98 ± 6.42
n-butanol	102.36 ± 0.94	490.52 ± 2.71

Results are expressed as mean values ± standard deviation (n = 3).

[a]TPC (Total phenolic content) values are expressed as mg gallic acid equivalent/g extract (mg GAE/g ext.).

[b]TFC (Total flavonoid content) values are expressed as mg rutin/g extract (mg RE/ g ext.).

Table 2: Antimicrobial activity of *Caesalpinia pulcherrima* extracts

	Clear Inhibition zone (□mm)			
Sample	*Candida albicans*	*Pseudomonas Aeruginosa*	*Staphylococcus aureus*	*Aspergillus niger*
100% MeOH	9	10	9	-
90% MeOH	8	9	8	-
85% MeOH	8	8	8	-
70% MeOH	13	8	10	-
Diethyl ether	-	-	-	-
Petroleum ether	-	-	-	-
Methylene Chloride	7	7	6	-
Ethyl acetate	7	8	8	-
n- butanol	9	8	6	-

The results of samples against *S. Aureus* = *Staphylococcus aureus* (G+ve bacteria); *C. albicans*= *Candida albicans* (Yeast); *A. Niger* = *Aspergillus niger* (Fungus); (-); inactive. Samples were dissolved in 2ml methanol and 100 micro liters were poured in 1ml-diameter cup. - Inhibition zone diameter of 250 µl of 20 mg/ml of different extracts

was higher when affected by 70% methanol extract. There was no inhibition in case of *Aspergillus Niger*. In previous study, the ethanolic extract of the dry fruit of *Caesalpinia pulcherrima* was tested for antimicrobial activity, which exhibited a broad spectrum of antimicrobial activity against *Escherichia coli* (Enteropathogen), *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* and this result give an indication for its strong antimicrobial activity against other strains beside the strains used in our study [36].

Antioxidant Activity: Due to the complex nature of the different phytochemical classes present in plants, the antioxidant capacities of plant extracts cannot be evaluated using a single method Khal and Dan [37]. In the present work, the TAC, RPAA, DPPH and methods were used to assess the antioxidant activities of plant extracts.

Total Antioxidant Capacity (TAC): The total antioxidant capacity of *Caesalpinia pulcherima* extracts were measured spectrophotometrically through phosphomolybdenum method which is based on the reduction of Mo (IV) to Mo (V) and the subsequent formation of green phosphate/Mo (V) compound with a maximum absorption at 695 nm. A high absorbance value of the sample indicates its strong antioxidant capacity. This method is a quantitative one, since the antioxidant capacity is expressed as the number of equivalent of ascorbic acid [17, 38, 39]. The results in (Table 3) showed that, all the tested extracts exhibited some degree of electron donation capacity, 90% methanol extract exhibited the highest antioxidant potency (444.4) followed by 85% methanol (401.2) the antioxidant potency of the extracts in TAC assay was in the following order 70% methanol (375.1) □ Butanol (364.1) □100% methanol (312) □ Ethyl acetate (311.6) □ methylene chloride (178.46) □ petroleum ether (144.02).

Scavenging Effect on 1, 1'-diphenyl-2-picrylhydrazyl Radical (DPPH): 1, 1'-diphenyl-2-picrylhydrazyl (DPPH) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimerize, as would be the case with most other free radicals [23].

Table 3: The total antioxidant capacity of the different extracts of *Caesalpinia pulcherrima* flower

Sample	Total antioxidant capacity (mg AAE /g ext.)
100% MeOH	312.00 ± 2.42
90% MeOH	444.43 ± 0.34
85% MeOH	401.22 ± 1.17
70% MeOH	375.12 ± 2.13
Diethyl ether	102.58 ± 2.14
Petroleum ether	144.02 ± 3.56
Methylene Chloride	178.46 ± 2.41
Ethyl acetate	311.60 ± 3.54
n- butanol	364.12 ± 4.02

Table 4: Free radical scavenging potential (DPPH) of the different extracts of *Caesalpinia Pulcherima* flower

Sample	Free radical scavenging potential DPPH SC_{50} [µg/ml]
100% MeOH	31.66 ± 1.01
90% MeOH	19.11 ± 0.35
85% MeOH	20.01 ± 0.14
70% MeOH	26.43 ± 0.18
Diethyl ether	71.98 ± 0.31
Petroleum ether	66.17 ± 0.72
Methylene Chloride	84.64 ± 2.21
Ethyl acetate	52.33 ± 0.26
n- butanol	28.12 ± 1.09

Results are means ± S.D., All experimental carried in triplicates (n=3). A higher DPPH radical-scavenging activity is associated with a lower SC_{50} value.

Table 5: Reducing power activity of the different methanolic extracts of *Caesalpinia Pulcherima* at concentration 200 µg/ml

Sample	Reducing Power antioxidant assay (RPAA)
100% MeOH	0.589
90% MeOH	0.679
85% MeOH	0.496
70% MeOH	0.375
Diethyl ether	0.189
Petroleum ether	0.263
Methylene Chloride	0.349
Ethyl acetate	0.512
n- butanol	0.733
Ascorbic acid	0.901

The delocalization also gives rise to the deep violet color, characterized by an absorption band in methanol solution centered at about 517 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color [40, 41]. The antioxidant effect is proportional to the disappearance of $DPPH^{\bullet}$ in test samples. The Violet color generally fades or disappears when an antioxidant is present in the medium. Results were reported as SC_{50}, which is the amount of antioxidant necessary to decrease the initial $DPPH^{\bullet}$ concentration by 50%. The lower the SC_{50}, the higher is the antioxidant power [42]. On the DPPH radical, the *Caesalpinia* extracts had significant scavenging effects with increasing concentration in the range of 1-200 µg/ml when compared with that of ascorbic acid, the DPPH activity of *Caesalpinia* was found to increase in dose dependent manner. The best free radical scavenging activity was exerted by 90% Methanol extract (SC_{50}= 19.11 µg/mL), which contained the highest amount of total phenolic. The lowest radical scavenging activity was exhibited by the methylene chloride extract (SC_{50}= 19.11µg/ml). The overall trend of the scavenging abilities of the extracts were in the following order 90% Methanol extract (19.11 µg/ml) >85% methanol (20.01 µg/ml) > 70% methanol (26.43 µg/ml) > Butanol (28.12 µg/ml) > ethyl acetate extract (52.3 µg/ml), > Petroleum ether (66.17 µg/ml) >diethyl ether (71.88 µg/ml) > methylene chloride (84.64 µg/ml) (Table 4).

Reducing Power Antioxidant Assay (RPAA): This method is based on the ability of substances, which have reduction potential, to react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form blue colored ferric ferrous complex $(Fe^{3+})_4[Fe^{2+}(CN^-)_6]^3$ that has an absorption maximum at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power of the sample, reducing power was reported as ascorbic acid equivalent per gm of dry sample. (Table 5) [35, 43]. The reducing power of the different extracts *C. Pulcherima* (OD value) was ranged from 0.189 to 0.733 with respect to ascorbic acid as standard.

Cytotoxic Activity: A simple animal that has been used to monitor lethality is the brine shrimp, *Artimia salina* Leach [26]. This procedure assumes that an animal that survived a given dose would also have survived any lower dose and, conversely, that an animal that died with a certain dose would have also died at any other higher does. Thus, the information from any one group can be added to that of the other groups in the range of doses tested [28]. The brine shrimp lethality bioassay is an efficient, rapid and inexpensive test that requires only a relatively small amount of the sample (2 -20 mg). This bioassay has a good correlation with cytotoxic activity in some human solid tumor and has led to the discovery of an active antitumor agent [44]. According to the standards of the National Cancer Institute (NCI), the results showed that *C. pulcherrima* gives cytotoxic effect. Butanol extract of *C. pulcherrima* exhibited the highest cytotoxic activity on brine shrimp larvae of *Artemia salina* with LC_{50}= 61.14 ppm followed by ethyl acetate extract at 99 ppm (Table 6) [35]. The other extracts showed medium cytotoxic effect.

Table 6: Mortality of Brine Shrimp larvae after 24 hrs of exposure to different extracts of *C. pulcherrima*

Sample	LC_{50} ± SE
100% MeOH	166 ± 3.13
90% MeOH	148 ± 4.25
85% MeOH	305 ± 1.29
70% MeOH	215 ± 2.80
Diethyl ether	275 ± 3.17
Petroleum ether	250 ± 4.15
Methylene Chloride	210 ± 2.15
Ethyl acetate	99 ±2.29
n- butanol	61.14 ± 4.63

Table 7: Inhibitory activity against Hepatocellular carcinoma cell of n-butanol extract

Sample conc. (ug)	Cell Viability %
100	38.79
50	49.12
25	64.39
12.5	80.45
6.25	94.12
3.125	98.66
0	100

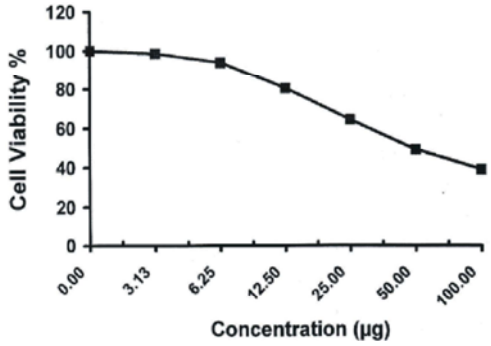

Fig. 1: Evaluation of the cytotoxicity of n-butanol extract of *C. pulcherrima* against HepG2 cell line

These results reveal these plants as potential antitumor agents. Inhibitory activity against hepatocellular carcinoma cell was detected, the n-BuOH fraction showed high cytotoxic activity toward the HepG2 cell line with IC_{50} = 48.6 µg/ ml (Table 7, Figure 1).

CONCLUSION

The present research work demonstrates that the flower part of *C. pulcherrima* has strong antioxidant, antimicrobial and cytotoxic activities and these activities are due to presence of certain bioactive chemical constituents, also these activities seem to depend on the content of phenolic constituents in each extract. On the basis of the results obtained in the present study, it is concluded that some tested extracts of *C. pulcherrima* flower, exhibit high antioxidant activity and antimicrobial effect against three strains as well as cytotoxic activity. These *in vitro* assays indicate that the plant extracts are a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

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