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Evaluation of antioxidant, antimicrobial and cytotoxic activities of *Terminalia citrina* Leaves

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ABSTRACT

Objective: To investigate antioxidant, antimicrobial and cytotoxic activities of methanol extract and its derived fractions (petroleum ether, carbon tetrachloride, dichloromethane and ethyl acetate) of leaves of *Terminalia citrina* plant belonging to the Combretaceae family. **Methods:** The antioxidant potential were evaluated in terms of total phenolic content, total flavonoid content, DPPH radical scavenging potential, ABTS scavenging potential, reducing power assay and total antioxidant capacity by specific standard procedures. The antimicrobial activity was evaluated using disc diffusion method while cytotoxic was evaluated by using brine shrimp lethality bioassay and compared with vincristine sulfate. **Results:** The methanolic leaf extract exhibited the highest flavonoid content and antioxidant capacity while ethyl acetate fraction exhibited the highest phenolic content, reducing power, DPPH and ABTS radical scavenging activity. Antioxidant activity was the most notable compared to the positive control and thus could be a potential rich source of natural antioxidant. In case of antimicrobial and cytotoxic activities, the extracts of the leaves does not showed remarkable activities against tested microorganisms and *Artemia salina* respectively. **Conclusions:** This study suggests that the leaves of *Terminalia citrina* possess antioxidant activity.

KEYWORDS: *Terminalia citrina*, Antioxidant, Antimicrobial, Cytotoxic activity

1. INTRODUCTION

Terminalia citrina (Bengali name: Haritaki, Family: Combretaceae) is a deciduous tree wide spread throughout the forest of Gazipur, Tangail, Sylhet, Chittagong, Rangamati and Chittagong hill tracts of Bangladesh. It is an important medicinal plant having various ethno pharmacological uses. Different parts of the plant are used for various ailments. The fruit is used in long-term fever, loss of appetite and as sexual stimulant in Bangladesh [1]; diarrhea, helminthes and other digestive disorders in Iran [2]. Its bark is diuretic and cardio tonic [3]. Seed is used in stomach aches and intestinal diseases [4]. The plant is also used in asthma, diarrhea, boils, burns, constipation, migraine, dental disease, haemoptysis, dizziness, bleeding hemorrhoids, eye disease, gastric hyperacidity, anemia, arthritis, hoarse voice, dysentery, pyrexia, infections, traumatic cuts, cardiac diseases, cough, hepatomegaly, urolithiasis and for life longevity in Myanmar [5]. A detailed literature survey revealed that seed of plant was reported to possess antioxidant properties [6] and five tannins identified as corilagin *(1) (3),* punicalagin *(2) (4),* 1,3,6-tri-O-galloyl-ß-D-glucopyranose *(3) (5),* chebulagic acid *(4) (6),* and 1,2,3,4,6-penta-O-galloyl-ß-D-glucopyranose *(5) (7)* was isolated from methanol extract of fruit [7]. However, no detailed pharmacological study has been reported in the literature. Therefore in the present investigation, we aimed to investigate antioxidant, antimicrobial and cytotoxic activities of *Terminalia citrina* leave.

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2. MATERIAL AND METHODS

2.1. Collection of Plant material

Terminalia citrina leaves were collected from Rangamati District, Bangladesh during the month of May 2013. The plant was identified and authenticated by Sardar Nasir Uddin, Senior Scientific Officer, Bangladesh National Herbarium Mirpur, Dhaka and a voucher specimen (Accession No: DACB 38094) was deposited there for future reference.

2.2. Extraction preparation

The leaves of the plant were collected in fresh condition. The dried and coarse powder (1000 g) was extracted with methanol (4.0 L) in an air tight flat bottomed container for 15 days at room temperature with occasional stirring. The extract was then filtered through a cotton plug followed by a Whatman No. 1 filter paper. The filtrate was con-

centrated using a rotary evaporator at low temperature and pressure to afford crude methanolic extract 50 g. A total of 20 g crude extract was then partitioned successively by four solvents of different polarity (petroleum ether, carbon tetrachloride, dichloromethane and ethyl acetate). The yield value in fractions of extract is petroleum ether (975 mg), carbon tetrachloride (835 mg), dichloromethane (640 mg) and ethyl acetate (590 mg).

2.3. Chemicals and reagents

1,1-diphenyl-2-picrylhydrazyl (DPPH), L-ascorbic acid, gallic acid, quercetin and Folin-Ciocalteu reagent were purchased from Sigma Chemical Co. (St. Louis, USA). Analytical grade dichloromethane, methanol, ethyl acetate and carbon tetrachloride were purchased from BDH, E Merck. Vincristine Sulphate was purchased from Cipla Ltd., Goa, India. All other chemicals and reagents were obtained commercially and were of analytical grade.

2.4. Phytochemical screening

The preliminary phytochemical group test was carried out by following standard procedure [8,9].

2.5. Antioxidant activity

2.5.1. Total phenolic content

The total phenols in extract and fractions of *T. citrina* were determined using Folin-Ciocalteu reagent [10]. About 0.5 mL of the each extract of concentration of 1 mg/mL was mixed with 5 mL Folin ciocalteu reagent (1:10 v/v distilled water) and 4 mL (75 g/L) of sodium carbonate. The mixture was allowed to stand for 30 min at room temperature in dark place after vortexing it for 15 seconds and the absorbance was measured at 760 nm against methanol as blank by using a UV-visible spectrophotometer. The total phenolic contents were expressed as gallic acid equivalent (GAE) in mg/g of dry weight.

2.5.2. Total flavonoid content

The total flavonoid in extract and fractions of *T. citrina* were measured by aluminum chloride ($AlCl_3$) colorimetric method [11]. About 0.5 mL methanol solution of each extract of concentration of 10 mg/mL was mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 mol/L potassium acetate and 2.8 mL of distilled water. The blank was prepared in similar fashion by replacing $AlCl_3$ with distilled water. Both sample and blank solution were filtered through double rings filter paper. After an incubation period of 30 min, the absorbance was measured at 415 nm against the blank by using a UV-visible spectrophotometer. The total flavonoid contents were expressed as quercetin equivalent in mg/g of dry weight.

2.5.3. DPPH free radical scavenging activity

The radical scavenging activities of each fractions of *T. citrina* were estimated using stable free radical of DPPH [12]. About 2.0 mL of methanol solution of each extract at different concentrations (5, 10, 20, 40, 60 80, 100 µg/mL) were mixed with 3.0 mL of DPPH methanol solution (20 µg/mL). After an incubation period of 30 min, the absorbance was measured at 517 nm against methanol as blank by using a UV-visible spectrophotometer. The radical scavenging activity (%) was calculated based on the following formula:

$$\text{DPPH scavenging activity (\%)} = [(A_B - A_T)/A_B] \times 100$$

Where A_B and A_T are the absorbance of blank and plant material, respectively.

The percentage scavenging activity of each extract was compared with ascorbic acid, the positive control. IC_{50} value of each extract was determined from the plotted graph of percentage DPPH neutralization vs concentration of extract, which was defined as the amount of antioxidant required to reduce the initial DPPH free radical concentration by 50%.

2.5.4. ABTS radical scavenging activity

The antioxidant activity was determined by ABTS radical cation described with some modifications [13]. ABTS radical cation was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate and the mixture was allowed to stand in the dark at room temperature for 16 h. In the moment of use, the ABTS solution was diluted with methanol to an absorbance of 0.70±0.02 at 734 nm. 1 mL of each sample with various concentrations (5, 10, 20, 40, 60, 80, 100 µg/mL) were added to 1 mL of ABTS solution and mixed vigorously. The reaction mixture was allowed to stand at room temperature for 6 min and the absorbance at 734 nm was immediately recorded. The ABTS scavenging effect was calculated as follows:

$$\text{ABTS scavenging activity (\%)} = [(A_B - A_T)/A_B] \times 100$$

Where A_B and A_T are the absorbance of blank and plant material, respectively.

The percentage scavenging activity of each extract was compared with ascorbic acid, the positive control. IC_{50} value of each extract was determined from the plotted graph of percentage ABTS neutralization vs concentration of extract, which was defined as the amount of antioxidant required to reduce the initial ABTS free radical concentration by 50%.

2.5.5 Reducing power assay

The method used to determine the reducing power with some modifications [14]. Different concentrations of the fractions (5, 10, 20, 40, 60,

80, 100 µg/mL) in 1 mL of methanol were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The mixture was then incubated at 50°C for 20 min and a 10% solution of trichloroacetic acid (2.5 mL) was added to it. It was then centrifuged at 3000 rpm for 10 min. The upper layer of the mixture (2.5 mL) was mixed with distilled water (2.5 mL) and 0.5 mL, 0.1% $FeCl_3$ and the absorbance of the mixture was measured at 700 nm with the same spectrophotometer. Increased absorbance of the reaction mixture indicated increased reducing power. All the determinations were carried out thrice and average of the results was taken. Ascorbic acid was used as the standard reference compounds in this study.

2.5.6 Total antioxidant capacity

The total antioxidant capacity was measured by spectrophotometeric method with slight modification [15]. At different concentration ranges, fractions were prepared in their respective solvents and combined in an eppendorf tube with 1mL of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate, 4mM ammonium molybdate mixture). The tubes were incubated for 90 min at 95ºC. The mixture was cooled to room temperature and the absorbance was read at 695 nm against blank. Ascorbic acid equivalents were calculated using standard graph of ascorbic acid. The experiment was conducted in triplicates and values are expressed as equivalents of ascorbic acid in mg per gm of extract.

2.6. Antimicrobial activity

A total of 16 reference microbial strains (five Gram-positive, eight Gram-negative and three fungi) were used as the test organism for the antimicrobial screening. The antimicrobial activity of fractions against the test organisms was performed by disc diffusion method using standard disc (5µg/disc) for comparison [16]. Ciprofloxacin was used as the standard disc for comparing antibacterial and antifungal activity. The test organisms were inoculated on 10 mL previously sterilized nutrient agar media, mixed thoroughly and transferred immediately to the sterile petri dish under an aseptic condition using a sterile loop. The paper discs containing the sample extract and standard disc were placed to the corresponding petri dish and were incubated for overnight at 37 °C. Clear zone of inhibition around the discs represented the presence of antimicrobial activity which was measured in millimeter (mm).

2.6.1. Collection of microorganisms

The microbial species used in the present study were *Bacillus cereus, Bacillus subtilis, Sarcina lutea, Staphylococcus aureus, Bacillus megaterium, Escherichia coli, Salmonella paratyphi, Salmonella typhi, Shigella boydii, Shigella dysenteriae, Pseudomonas aeruginosa, Vibrio mimicus, Vibrio parahemolyticus, Candida albicans, Aspergillus niger* and *Sacharomyces cerevacae*. These were collected as pure cultures from the Institute of Nutrition and Food Sciences, Dhaka University, Dhaka, Bangladesh.

2.7. Determination of cytotoxicity

The cytotoxic potentiality of all the extracts of *T. citrina* were performed on brine shrimp nauplii using Mayer's method [17,18]. The eggs of brine shrimp (*Artemia salina* Leach) were collected and hatched in a tank containing 1 L of simulated seawater at a temperature around 37 °C and pH 8.4 with constant oxygen supply. Two days were allowed to hatch and mature the nauplii. Pure DMSO solutions of the extracts were applied to *Artemia salina* in a one-day *in vivo* assay. About 4 mg of each extracts was dissolved in DMSO and solutions with varying concentrations (400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781 µg/mL) obtained by serial dilution technique. The prepared test solutions were added to the pre-marked vials containing 10 live brine shrimp nauplii in 5 mL simulated seawater and incubated for 24 h. After incubation period, the vials were examined using a magnifying glass in order to count the number of survived nauplii in each vial. From this data, the lethality percent of the brine shrimp nauplii was calculated for each concentration. The median lethal concentration LC_{50} of each tested sample was calculated from the plotted graph of percentage of the shrimp mortality vs logarithm of the sample concentration, which was defined as the amount of extract required to kill 50% of brine shrimps within 24 h of exposure respectively.

2.8. Statistical analysis

All values were expressed as mean±SD of three parallel determinations. Regression analysis was carried out for analyzing the data obtained from brine shrimp lethality bioassay.

3. RESULTS

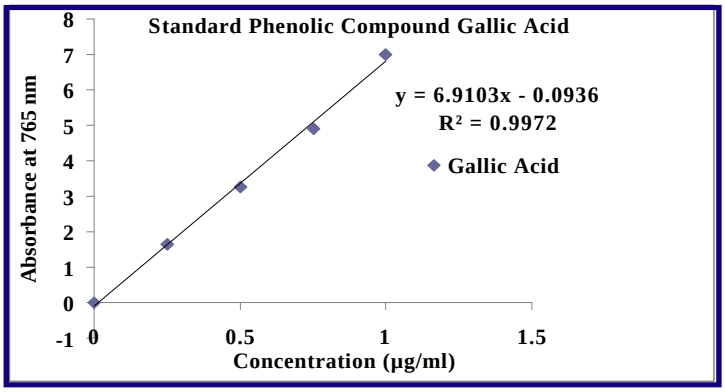
3.1. Phytochemical screening

The phytochemical screening test showed the presence of alkaloids, flavonoids, tannins, reducing sugar and carbohydrates in the leaves of *T. citrina*.

3.2. Total phenolic and flavonoid contents

Table 1 provides information about the total phenolic and flavonoid contents in the extract and fractions of leaves of *T. citrina*. The highest phenolic and flavonid contents were found in the ethyl acetate fraction and methanolic extract of leaves of *T. citrina* which amounted to (327.22±9.87) GAE/g of dry weight and (99.51±0.221) mg quercetin

equivalents/g of dried weight, respectively. Carbon tetrachloride fraction was found to contain the lowest phenolic and flavonid contents.



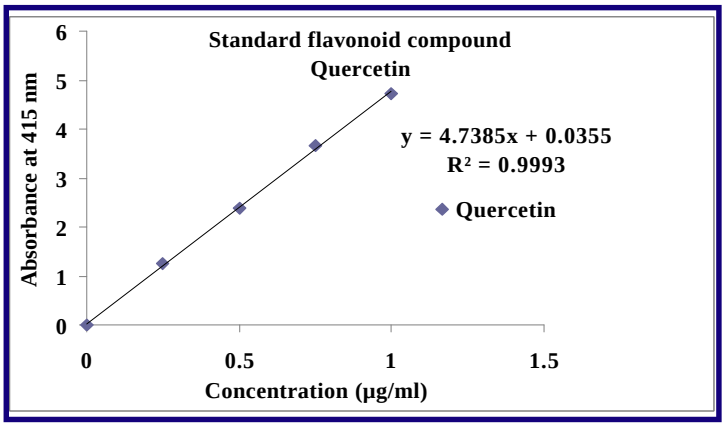


Figure 1. Standard calibration curve for determination of total phenolic and flavonoid contents

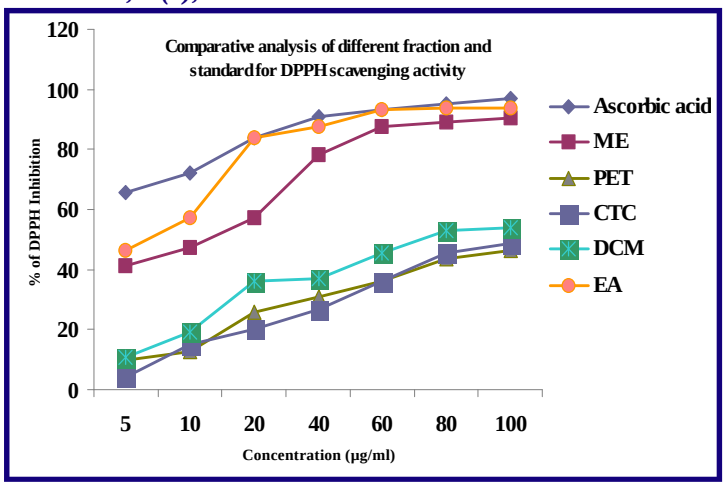
Table 1. Total Phenolic and flavonoid contents of methanol extract and fractions of *T. citrina* leaves

Name of Extract	Total phenolic content (mg GAE/ g of dry ext.)	Total flavonoids content (mg QE/g of dry ext.)
Methanol extract	190.23±5.24	99.51±0.221
Petroleum ether fraction	27.76±0.35	0.66±0.02
Carbon tetrachloride fraction	18.39±0.15	0.28±0.01
Dichloro methane fraction	52.31±0.43	4.56±0.07
Ethyl acetae fraction	327.22±9.87	39.40±0.16

Values are expressed as mean±SD (n=3)

3.3. DPPH free radical scavenging activity

The percentage of DPPH neutralization of methanol extract and fractions were found to be concentration dependent. Ethyl acetate fraction produced the maximum free radical scavenging activity with IC_{50} value of 7.84±0.19 µg/mL, which was comparable value to that of reference antioxidant of the test. Among all leaf extracts, carbon tetrachloride fraction of leaf was found to produce weakest free radical scavenging activity. Figure 2 represents the percentage of DPPH neutralization activity of all extracts considered in the study.

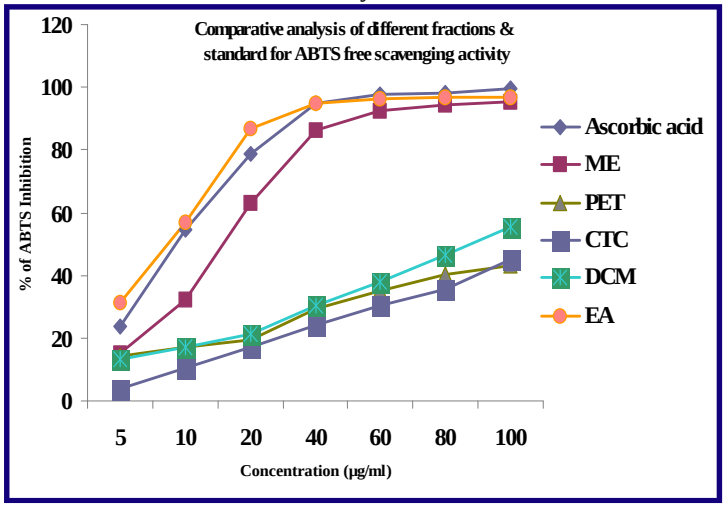


ME – Methanol extract, PET – Petroleum ether ,CTC – Carbon tetrachloride, DCM – Dichloromethane, EA – Ethyl acetate

Figure 2. DPPH scavenging activity of *T. citrina* leaves

3.4. ABTS Radicals Scavenging Activity

ABTS assay is used in evaluating total antioxidant power of single compounds and complex mixtures of various plants [19]. Specific absorbance at 734 nm can be used in both organic and aqueous solvents as an index reflecting the antioxidant activity [20]. The percentage of ABTS neutralization of methanol extract and fractions were found to be concentration dependent. Ethyl acetate fraction produced the maximum free radical scavenging activity with IC_{50} value of 7.84±0.17 µg/mL, which was lower than that of reference antioxidant of the test. Among all leaf extracts, carbon tetrachloride fraction of leaf was found to produce weakest free radical scavenging activity. Figure 3 represents the percentage of ABTS neutralization activity of all extracts considered in the study.



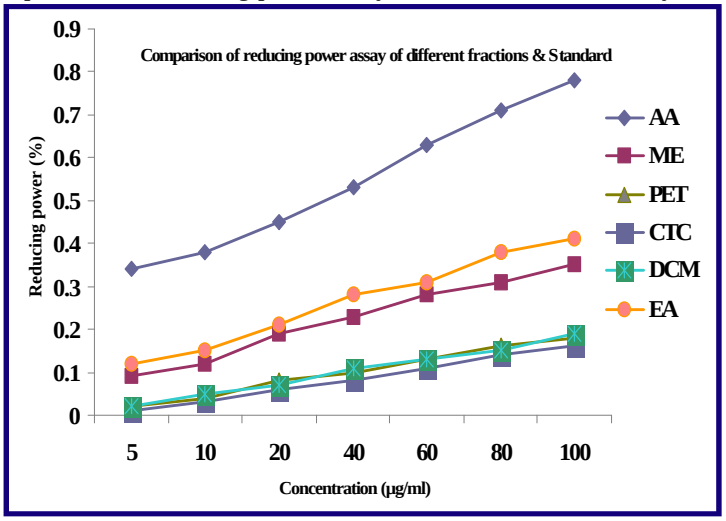
ME – Methanol extract, PET – Petroleum ether ,CTC – Carbon tetrachloride, DCM – Dichloromethane, EA – Ethyl acetate

Figure 3. ABTS scavenging activity of *T. citrina* leaves

3.5. Reducing power assay

The reducing power of all extracts was also found to be concentration dependent. Like percentage of DPPH neutralization activity,

ethyl acetate fraction provided the most intense reducing power with value of 0.41±0.038 at 100 µg/mL concentration, which was comparable to that of standard antioxidant used in the test. Carbon tetrachloride fraction produced the least reducing power assay. Figure 4 represents the reducing power assay of all extracts in the study.



ME – Methanol extract, PET – Petroleum ether ,CTC – Carbon tetrachloride, DCM – Dichloromethane, EA – Ethyl acetate

Figure 4. Reducing potential of *T. citrina* leaves

3.6. Total antioxidant capacity

Total antioxidant capacity of all extracts is expressed in Table 2. The most powerful antioxidant activity was recorded in methanol extract which amounted to (10.17±0.02) mg AAE equivalents/g of dry weight while carbon tetrachloride fraction was found to provide the least antioxidant activity which amounted to (0.81±0.01) mg AAE equivalents/g of dry weight.

Table 2. Total antioxidant capacity of methanol extract and fractions of *T. citrina* leaves

Name of Extract	Absorbance at 695nm	Total Antioxidant Capacity (AAE/gm)
Methanol extract	0.5260±0.091	10.17±0.02
Petroleum ether	0.0846±0.005	1.38±0.02
Carbon tetrachloride	0.0554±0.001	0.81±0.01
Dichloromethane	0.0921±0.018	1.54±0.04
Ethyl acetate	0.4945±0.017	9.55±0.05

Values are expressed as mean±SD (n=3)

3.7. Antimicrobial activity

The results of different extracts of *T. citrina* with disc diffusion method are shown in Table 3. The antimicrobial activity of all test fractions was tested using concentration 400 µg/disc. No zone of inhibition was noticed against the growth of tested microorganisms except ethyl acetate fraction.

Table 3. Antimicrobial activity of test samples of *T. citrina* leaves

Test organism	Diameter of zone of inhibition(mm)					
	STD	ME	PET	CT	DCM	EA
Gram positive Bacteria						
Bacillus cereus	46	-	-	-	-	8
Bacillus megaterium	47	-	-	-	-	8
Bacillus subtilis	46	-	-	-	-	9
Sarcina lutea	44	-	-	-	-	8
Staphylococcus aureus	44	-	-	-	-	8
Gram negative Bacteria						
Escherichia coli	44	-	-	-	-	8
Pseudomonas aeruginosa	44	-	-	-	-	8
Salmonella paratyphi	45	-	-	-	-	8
Salmonella typhi	44	-	-	-	-	9
Shigella boydii	45	-	-	-	-	8
Shigella dysenteriae	44	-	-	-	-	8
Vibrio mimicus	45	-	-	-	-	7
Vibrio parahemolyticus	45	-	-	-	-	8
Fungi						
Aspergillus niger	45	-	-	-	-	8
Candida albicans	45	-	-	-	-	8
Sacharomyces cerevacae	45	-	-	-	-	7

'-' Indicates no zone of inhibition,STD - Ciprofloxacin,ME – Methanol extract,PET – Petroleum ether ,CTC – Carbon tetrachloride,DCM – Dichloromethane,EA – Ethyl acetate

3.8. Cytotoxic activity

In brine shrimp lethality bioassay, percentage of mortality increased gradually with the increase in concentration of the test samples. From the results (Table 4) it was revealed that petroleum ether fraction gave maximum cytotoxicity with the lower LC_{50} value of 10.94 g/mL. In comparison to positive control (Vincristine sulphate), the cytotoxic potentiality exhibited by *T. citrina* extracts were very negligible.

Table 4. LC_{50} values of the test samples of *T. citrina* leaves

Test sample	Regression line	R^2	LC_{50} (µg/mL)
VS	y=33.223x +58.787	0.9581	0.544
ME	y=35.233x - 2.9501	0.9384	31.831
PET	y=33.622x +15.059	0.9593	10.945
CTC	y=30.803x +10.575	0.8991	19.050
DCM	y=26.374x -3.8998	0.9472	110.578
EA	y=34.226x -10.694	0.8973	59.337

VS - Vincristine sulphate,ME – Methanol extract,PET – Petroleum ether CTC – Carbon tetrachloride,DCM – Dichloromethane,EA – Ethyl acetate

4. DISCUSSION

Natural products derived from plants have been used in folklore medicine to treat different diseases. So with the objective to contribute to the knowledge of the medicinal flora and considering the use of this plant, a pharmacological study of the methanol extract and its frac-

tions of the leaves of *T. citrina* were done. To our knowledge, this is the first time that the antioxidant, antimicrobial and cytotoxic activities in experimental models is reported.

Plant materials rich in phenolics are increasingly being used in the food industry because they retard oxidative degradation of lipids and improve the quality and nutritional value of food [21]. Phenolic compounds are considered secondary metabolites and these phytochemical compounds derived from phenylalanine and tyrosine occur ubiquitously in plants and are diversified [22]. The ethyl acetate extract exhibited the highest total phenolics content, whereas the contents obtained with residual carbon tetrachloride fraction were much smaller that is in agreement with other reports [23]. Phenolic compounds of plants are also very important because their hydroxyl groups confer scavenging ability.

Phenolic compounds of plants fall into several categories; chief among these are the flavonoids which have potent antioxidant activities [24]. Flavonoids are naturally occurring in plants and are thought to have positive effects on human health. Studies on flavonoidic derivatives have shown a wide range of antibacterial, antiviral, anti inflammatory, anticancer, and anti-allergic activities [25,26]. Flavonoids have been shown to be highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various free radicals [27] implicated in several diseases. So comparable with the findings in the literature for other extracts of plant products [28] our results suggested that phenolic acids and flavonoids may be the major contributors for the antioxidant activity of various soluble fractions of *T. citrina* and the contents of phenolics or flavonoids exhibited significant correlation. Several techniques have been used to determine the antioxidant activity *in vitro* in order to allow rapid screening of substances since substances that have low antioxidant activity *in vitro*, will probably show little activity *in vivo* [24]. Free radicals are known to play a definite role in a wide variety of pathological manifestations. Antioxidants fight against free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms [29].

The scavenging potential of all five extracts of *T. citrina* was appraised through investigating their DPPH reduction against the positive control (L-ascorbic acid). DPPH radical loses its chromophore upon receiving proton from hydrogen donor. Consequently, increased concentration of phenolic compounds or number of hydroxyl group on aromatic ring boosts DPPH radical scavenging activity [30,31]. The antioxidative as well as the scavenging potential of extract is directly proportional to the DPPH reduction. The more antioxidants found in extract, the more DPPH reduction will occur. Higher DPPH reduction is associated with greater scavenging potential. Since all extracts showed dose dependent DPPH scavenging activity, these extracts may exert more pronounced and significant free radical scavenging activity. The antioxidant potential of the extracts measured by DPPH scavenging method was also expressed as 50% inhibitory concentration, IC_{50} values. Ethyl acetate extract was found to have the lowest IC_{50} value among the other extracts. The result of our study indicates a strong relationship between phenolic content and DPPH scavenging as well as antioxidant activities, suggesting that the phenolic compounds are probably responsible for the antioxidant activity of *T. citrina*.

DPPH is frequently used in the determination of free radical scavenging ability; however, it has the limitation of colour interference and sample solubility. Therefore, the free radical scavenging ability of the plant extracts were further studied using a moderately stable nitrogen-centered radical species: ABTS (2,2-azinobis (3-ethylbenzothiazoline- 6-sulfonate). The ABTS radical based model of free radical scavenging ability has the advantage of being more versatile as both non-polar and polar samples can be assessed and spectral interference is minimized as the absorption maximum used is 734 nm, a wavelength not normally encountered with natural products [32]. ABTS and DPPH free radical scavenging abilities follow the same trend. This was found to be; carbon tetrachloride< petroleum ether < dichloromethane< methanol< ethyl acetate in ascending order.

In reducing power assay, the yellow colour of the test solution changes to green depending on the reducing power of the test specimen. The presence of the reductants in the solution causes the reduction of the Fe^{3+}/ ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by absorbance measurement at 700 nm. Previous reports suggested that the reducing properties have been shown to exert antioxidant action by donating of a hydrogen atom to break the free radical chain [33]. Increasing absorbance at 700 nm indicates an increase in reducing ability. The antioxidants present in the fractions of *T. citrina* caused their reduction of Fe^{3+}/ ferricyanide complex to the ferrous form, and thus proved the reducing power. Like DPPH scavenging activity, ethyl acetate was found to have the highest reducing potential value among the other extracts.

The antioxidant capacity of the fractions was measured spectrophotometrically through phosphomolybdenum method, based on the reduction of Mo (VI) to Mo (V) by the test sample and the subsequent formation of green phosphate/Mo (V) compounds with a maximum absorption at 695 nm. The present study demonstrated that methanol extract exhibited the highest antioxidant capacity for phosphomolybdate reduction. Recent studies have shown that many

flavonoid and related polyphenols contribute significantly to the phosphomolybdate scavenging activity of medicinal plants [34,35].

Few studies have been done on the antibacterial and antifungal properties of *T. citrina*. Due to the reported development of resistance by bacteria and fungi to various commercially available antimicrobial agents, the leaves extract of plants are potential sources of new compounds which may be developed as effective drugs against microorganisms [36]. In our study, antimicrobial activity of various leaves extracts of *T. citrina* is evaluated by disc diffusion method. Single dose of extracts were used in the test and compared with the positive control. All extracts of leaves don't exhibit any antimicrobial activity against tested microorganisms. Nevertheless, the inhibition zone produced by the commercially available positive control was larger than those produced by the extracts. The presence of very minute concentrations of bioactive compounds in the plant extract [37] and variation of collection place & time and extraction process of the plant may also contribute to the poor antimicrobial activity [38].

Since any compound or extract can exert antimicrobial and antioxidant activity as a result of its toxic effects on the cells, determination of the toxic effect of antimicrobial and antioxidant agents on host cell is mandatory [39]. For this purpose, brine shrimp lethality test was performed. From the results of brine shrimp lethality bioassay, it can be concluded that the all extracts of *T. citrina* did not show any apparent *in vitro* toxicity compared to positive control [40]. The presence of minor amount of bioactive cytotoxic compound in the extracts may contribute to the weak result.

In conclusion, the current study illustrates the leaves of *T. citrina* should be regarded as a valuable source of material for human health, as an antioxidant agent. Further studies are desirable to characterize and isolate the unknown underlying components in order to establish their pharmacological properties which could provide valuable lead compounds in the respective therapeutic area.

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