

## Pharmacological Activities of Natural Products Derived from *Clerodendrum fortunatum* L

Rashid MA\*, Jubair MH and Atiqur Rahman

Department of Applied Chemistry and Chemical Engineering, Islamic University, Kushtia-7003, Bangladesh

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\*Corresponding author: Dr. Md. Mamun Al Rashid

### Abstract

### Original Research Article

The antioxidant property of the methanol and ethanol extracts from various parts of *Clerodendrum infortunatum* L. was examined *in vitro*. Antioxidant activity was evaluated by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay. The free radical scavenging activity of ethyl acetate fraction was superior to all other fractions ( $IC_{50} = 16.45 \mu\text{g/ml}$ ), which was higher than synthetic antioxidant butylate dhydroxy anisole, BHA, ( $18.27 \mu\text{g/ml}$ ). Furthermore, the amount of total phenolic compounds was determined and its content in EtOAc fraction (12.25%) was the highest as compared to other extract or fractions. The essential oil and organic extracts also showed potent antibacterial activities against the tested bacteria such as *Escherichia coli* ATCC 35218, Methicillin-resistant *Staphylococcus aureus* (isolate), *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853 and *Enterococcus faecalis* ATCC 291212. Our study suggests that natural products derived from *Clerodendrum fortunatum* L. have the potential to be used as food preservatives.

**Keywords:** *Clerodendrum infortunatum* L.; Antioxidant activity; Antibiotic activity; DPPH.

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## INTRODUCTION

There is a growing interest in research on food components such as phenolic compounds because of their possible linkage to health benefits, e.g., reduction of heart disease and cancer, based on their antioxidant activity [1]. Many medicinal plants contain large amount of antioxidants such as polyphenols, which have an important role in preventing a variety of stress-related diseases and aging because these are closely related to the active oxygen and lipid peroxidation [2]. Antioxidants have been used for the prevention and treatment of free radical-related disorders [3]. However, there have been concerns about synthetic antioxidants such as butylate dhydroxy anisole (BHA) and butylate dhydroxy toluene (BHT) because of their possible activity as promoters of carcinogenesis [4]. Consequently, there is a scientific interest to find naturally occurring antioxidants for using as natural preservative ingredients in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their carcinogenicity [5].

Among the plants known for medicinal value, the plants of genus *Clerodendrum* belonging to family Lamiaceae are very important for their therapeutic

potentials. *C. infortunatum* L. known as Vati flower in Bengali.

Several ethno botanical surveys show that *C. infortunatum* was among the plants reported to be used traditionally to treat bacterial infections such as enteric diseases i.e., diarrhea, dysentery and other gastrointestinal infections; upper respiratory tract infections associated with coughing pneumonia, asthma and bronchitis; urogenital infections including sexually transmitted diseases, skin infections (dermatitis, eczema, scabies), wounds and ulcers; headache, ophthalmic, insect bites, nasal bleeding, stroke, measles, paludism; and bacterial fevers such as typhoid fever and diabetes and veterinary problems [6,7]. It is also used in the treatment of epilepsy, shigellosis, trypanosomiasis, convulsion, pile and anaemia [8]. It is also implicated in the oral hygiene and veterinary [9, 10]. Comprehensive biological activities of *C. infortunatum* have been reviewed [7] and it is associated with a wide variety of biological activities [11-14].

For the production of energy to fuel through biological processes oxidation is essential in many living organisms. However, oxygen-centered free

radicals and other reactive oxygen species (ROS), which are continuously, produced *in vivo*, result in cell death and tissue damage. The role of oxygen radicals has been implicated in several diseases, including cancer, diabetes and cardiovascular diseases, ageing, etc. [15]. Antioxidant has been used for the prevention and treatment of free radical related disorders. Antioxidants halt the free radical chain reactions. Some antioxidants are themselves free radicals, donating electrons to stabilize and neutralize the dangerous free radicals. Other antioxidants work against the molecules that form free radicals, destroying them before they can begin the domino effect that leads to oxidative damage. However there have been concerns about synthetic antioxidants such as ascorbic acid because of possible activity as promoters of carcinogenesis. Synthetic antioxidants, such as butylate dhydroxy anisole (BHA) and butylate dhydroxy toluene (BHT), also have restricted to use in foods as they are suspected to be carcinogenic [16]. Therefore, the importance of searching natural antioxidants has greatly increased in recent years [17, 18]. The pharmaceutical industry is undertaking the rapid development and use of natural antioxidants, especially those of plant origin, to replace synthetic drug. Moreover, essential oils are plant secondary metabolites, mainly monoterpenes, sesquiterpenes and their corresponding oxygenated derivatives, which have been showed various pharmacological effects, such as antimicrobial, antioxidant, spasmolytic, carminative, hepato protective, antiviral and anti-carcinogenic effects [19,20]. Although it remains unclear which of the compounds, of medicinal plants are the active ones, phenolics recently have received increasing attention because of some interesting new findings regarding their biological activities [21]. From pharmacological and therapeutic points of view, the antioxidant properties of phenolics, such as free radical scavenging and inhibition of lipid peroxidation, are the most crucial. Even though a variety of herbs and plants are known to be the sources of phenolic compounds, studies isolating phenolics and evaluating their anti oxidative effects have rarely been carried out [21, 22].

## MATERIALS AND METHODS

Samples (*Clerodendrum fortunatum* L) were collected from Kawraid, Sreepur, Gazipur area of the Bangladesh during May to June, 2017. The plants were identified by Ahasan Kabir Rana, Associate Professor, Botany, Kushtia Govt. College, Kushtia, Bangladesh on the basis of morphological features and the voucher specimen no. 40701 have been deposited in the Bangladesh National herbarium, Dhaka.

The air-dried flower of *Clerodendrum infortunatum* L powdered (50 g) were extracted with ethanol and methanol separately at room temperature for 7 days and the solvents were evaporated by vacuum rotary evaporator. The extraction process yielded in methanol (4.5 g) and ethanol (6.4 g) extracts.

## ANTIOXIDANT ASSAY

### Free radical scavenging capacity

The antioxidant activity of the methanol and ethanol extract from various parts of *Clerodendrum infortunatum* L. were measured on the basis of the scavenging activities of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical [23] (Cuendet, Hostettmann, &Potterat, 1997). Various concentrations of 100 µl of test extract or fractions were added to 3 ml of a 0.004% (w/v) methanol solution of DPPH. After 30 min of incubation period in the dark at room temperature, the absorbance was measured against a blank at 517 nm. Inhibition of free radical DPPH in percent (%) was calculated by the formula:

$$\text{Percentage inhibition (\%)} = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100$$

Where,  $A_{\text{blank}}$  is the absorbance of the control reaction (containing all reagents except test compound), and  $A_{\text{sample}}$  is the absorbance of the test compound.  $IC_{50}$  values (concentration of sample required to scavenge 50% of free radicals) were calculated from the regression equation, prepared from the concentration of the extract or fractions and percentage inhibition of free radical formation/percentage inhibition DPPH was assayed. Synthetic antioxidant reagents, butylate dhydroxy anisole (BHA) and L-ascorbic acid, were used as positive controls and all tests were carried out in triplicate.

### Determination of total phenolics

Total phenolic constituent of the aforementioned extracts were determined by Folin-Ciocalteu reagent in alkaline medium [24] and was expressed as gallic acid equivalents (GAE). Different concentrations of gallic acid were prepared in 80% methanol. 100 µl test sample (from a range of concentrations) was taken in a cuvette, then 1 ml of distilled water and 500 µl (1/10 dilution) of the Folin-Ciocalteu reagent was added, and cuvette was shaken thoroughly. After 1 min, 1500 µl of 20% sodium carbonate ( $Na_2CO_3$ ) solution was added. The final mixture was shaken and then incubated for 2 h in the dark at room temperature. The absorbance of samples was measured at 760 nm and the results were expressed in mg of gallic acid/ g (GAE) of dry weight of samples.

### Antibacterial activity assay

#### Bacterial strains

*Escherichia coli* ATCC 35218, Methicillin-resistant *Staphylococcus aureus* (isolate), *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853 and *Enterococcus faecalis* ATCC 291212 were used in this study. These bacteria were cultured using Nutrient Broth (LabM Limited, UK) and Bacteriological Agar No.1 (LabM Limited, UK) at 37°C for 24 h. Microorganisms are kindly provided by Assoc. Prof. Dr. Sezer OKAY, Cankiri Karatekin University, Faculty of Science Department of Biology, Cankiri Turkey.

### Disc diffusion assay

The essential oil was diluted 1:5 (v/v) with methanol and sterilized by filtration using 0.22 µm sterile Millipore filter (Millipore Corp., Billerica, MA, USA) and aliquots of 10 µL were spotted onto the sterile What man No. 1 filter paper discs (6 mm diameter); while the extracts were dissolved in the same solvent used for their extraction and 10 µL of 30 mg mL<sup>-1</sup> of each organic extract (300 µg disc<sup>-1</sup>) was applied on the filter paper discs and placed on the inoculated LB agar medium. Then the antibacterial test was carried out as described [20] using 100 µL of standardized inoculums suspension containing 10<sup>7</sup> CFU mL<sup>-1</sup> of bacteria. Negative controls were prepared using the same solvents employed to dissolve the samples. Standard antibiotic streptomycin (10 µg disc<sup>-1</sup>) from Sigma-Aldrich Co., St. Louis, MO, USA) was used as positive control for the tested bacteria. The plates were incubated micro aerobically at 37 °C for 24. Antibacterial activity was evaluated by measuring the diameter of the zones of inhibition against the tested

bacteria. Each assay in this experiment was replicated three times.

### Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the essential oil and various extracts was assessed according to Chandrasekaran and Venkatesalu [21]. Active cultures for MIC determination were prepared by transferring a loopful of cells from the stock cultures to flasks and inoculated in LB medium and incubated at 37 °C for 24 h. The samples were incorporated into LB broth medium to get the final concentration ranging from 0 to 1000 µg mL<sup>-1</sup>. Finally, 20 µL inoculums of each bacteria strain (10<sup>7</sup> CFU mL<sup>-1</sup>) was transferred to each tube and the tests were performed in a volume of 2 mL. The control tube contained only organisms and not the samples. The culture tubes were incubated at 37 °C for 24 h. The lowest concentration of the test samples, which did not show any visual growth of tested organisms after macroscopic evaluation, was determined as MIC, which was expressed in µg mL<sup>-1</sup>.

## RESULTS AND DISCUSSION

### Antioxidant Results

**Table-1: Total phenolic compounds of *Clerodendrum infortunatum* L. extracts**

Extracts	Total phenolic(mg GAE/g dw) <sup>a</sup>
Methanol extract	71.63 ± 2.2
Hexane fraction	24.87 ± 1.8
Chloroform fraction	39.43 ± 0.8

<sup>a</sup>Values are given as the mean ± S.D. of triplicate experiments.

**Table-2: DPPH scavenging activities of *C. infortunatum* L. in methanolic and ethanolic extract**

Extract	Used part	Conc.µg/ml	Inhibition%	IC <sub>50</sub> Value
Methanol Extract	Leaf	100	71.56±1.2	10.35
		150	85.21±1.5	
		200	96.12±1.1	
	Stem	100	69.38±1.2	15.27
		150	80.47±1.3	
		200	92.14±1.4	
	Root	100	55.15±1.5	72.24
		150	69.67±0.5	
		200	77.64±1.5	
Ethanol Extract	Leaf	100	67.23±1.7	22.41
		150	81.45±1.6	
		200	90.54±1.4	
	Stem	100	65.14±1.3	36.05
		150	81.15±1.1	
		200	90.54±0.5	
	Root	100	48.12±0.7	104.43
		150	61.11±1.2	
		200	68.14±1.2	

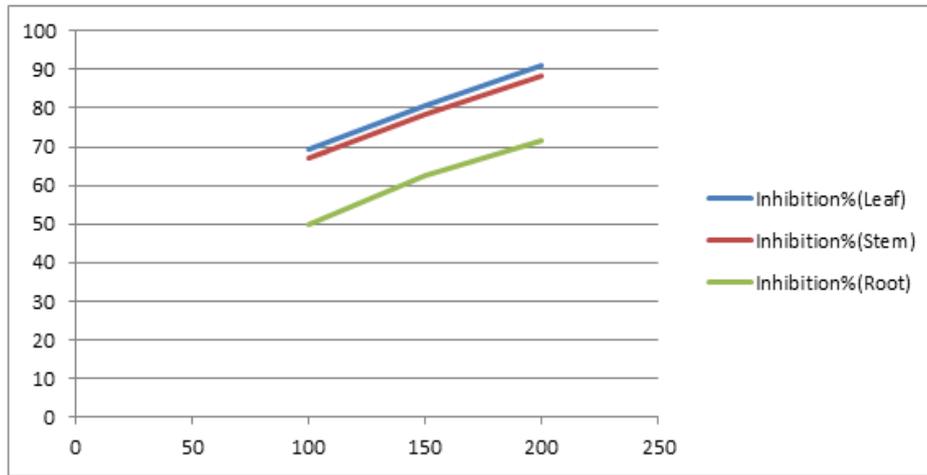


Fig-1: DPPH scavenging activities of *Clerodendrum infortunatum* L. in methanolic extract.

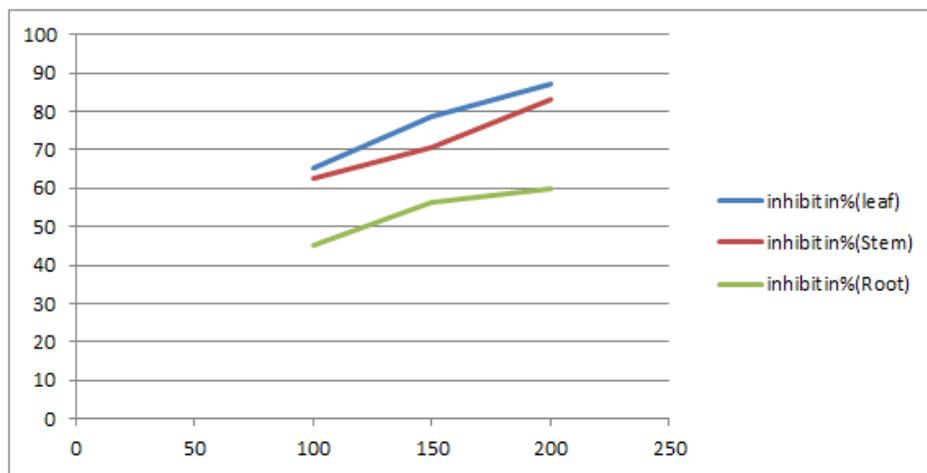


Fig-2: DPPH scavenging activities of *Clerodendrum infortunatum* L. in ethanolic extract

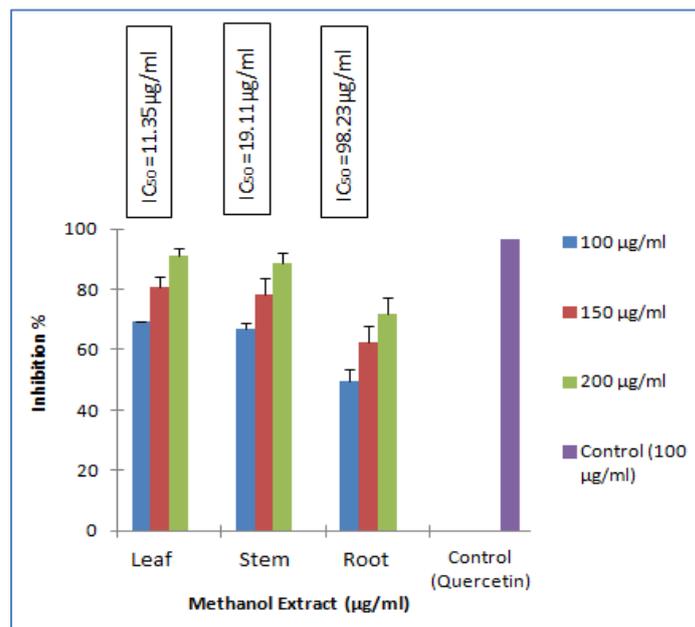


Fig-3: DPPH scavenging activities of *Clerodendrum infortunatum* L. in methanolic extracts

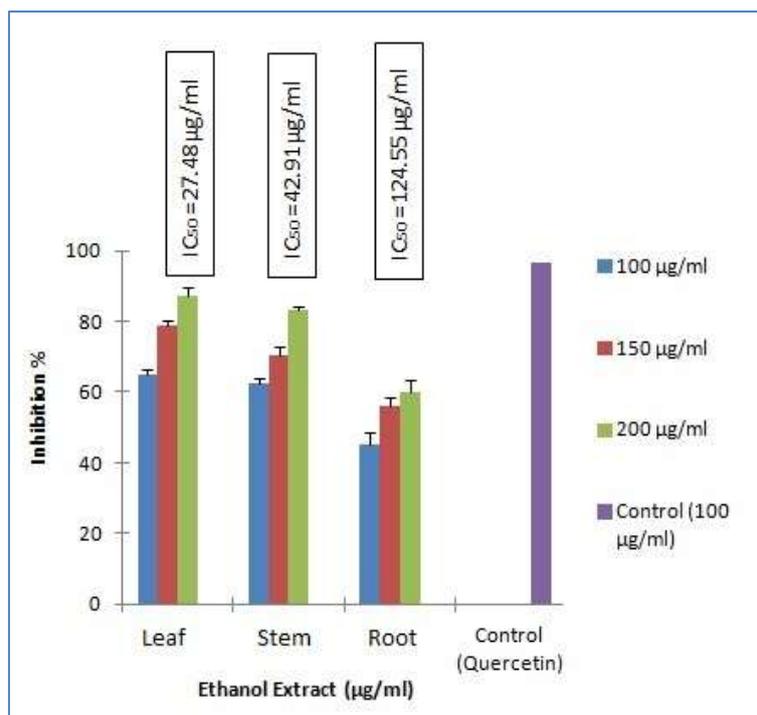


Fig-4: DPPH scavenging activities of *Clerodendrum infortunatum* L. in ethanolic extracts

Antioxidant has been used for the prevention and treatment of free radical related disorders. However there have been concerns about synthetic antioxidants such as ascorbic acid because of possible activity as promoters of carcinogenesis. There is a scientific interest to find naturally occurring antioxidants for use in foods to replace synthetic antioxidants.

In this study we found that crude extracts of *Clerodendrum infortunatum* L. Enrich in phenolic compounds (table !.) have strong DPPH scavenging activities. The antioxidant effect of these extracts is due to the presence of phenolic or alcoholic components [25]. The antioxidant activity is to be mainly due to their redox properties which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides. The DPPH free radical scavenging activity of leaves, stems and roots extracts have been shown in Fig.1.2,3 and Fig.4. The IC<sub>50</sub> values were compared with the IC<sub>50</sub> value of Quercetin (positive control). A lower IC<sub>50</sub> value indicates a greater antioxidant activity. In methanol and ethanol the IC<sub>50</sub> values of leaves of *Clerodendrum infortunatum* L. were recorded 11.35 µg/ and 27.48 µg/ ml, respectively (table2.).

Free radical scavenging activity of methanol and ethanol extracts measured by DPPH assay, is shown in Fig. 1. Their activity of the plant extracts is concentration dependent and lower IC<sub>50</sub> value reflects better protective action. The IC<sub>50</sub> values of methanol and ethanol extracts were recorded in the range of 10.35 to

104.43 µg/ml. Methanol extract exhibited stronger DPPH scavenging activity than ethanol extract. The free radical scavenging activity of methanol extract (IC<sub>50</sub> = 10.35 µg/ml) was superior to all other extract. The IC<sub>50</sub> value of methanol extract (IC<sub>50</sub> = 10.35 µg/ml) was lower than synthetic antioxidant, butylated hydroxyanisole (BHA) (18.27 µg/ml). Therefore, methanol extract showed higher activity than butylated hydroxyanisole. The strongest activity of methanol extract may be related to its higher phenolic content (71.63 mg GAE/g) as measured by gallic acid test (Table 1).

#### Antibacterial activity

The isolated essential oil and organic extracts (chloroform, EtOAc and MeOH) were dissolved in DMSO to make the required concentration (10 µL disc<sup>-1</sup> correspond to 50 µg disc<sup>-1</sup>). Then the antibacterial test was carried out as described<sup>20</sup> using 100 µL of standardized inoculum suspension containing 10<sup>7</sup> CFU mL<sup>-1</sup> of bacteria including *Escherichia coli* ATCC 35218, Methicillin-resistant *Staphylococcus aureus* (isolate), *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853 and *Enterococcus faecalis* ATCC 291212. As shown in Table 16, all these compounds displayed potent antibacterial activities against all the tested bacteria. Standard antibiotics; tetracycline (30 µg disc<sup>-1</sup>), streptomycin (30 µg disc<sup>-1</sup>) and erythromycin (15 µg disc<sup>-1</sup>) were used as positive controls, whereas DMSO was used as negative control. As a blind control, DMSO did not show any effect of this study (data not shown).

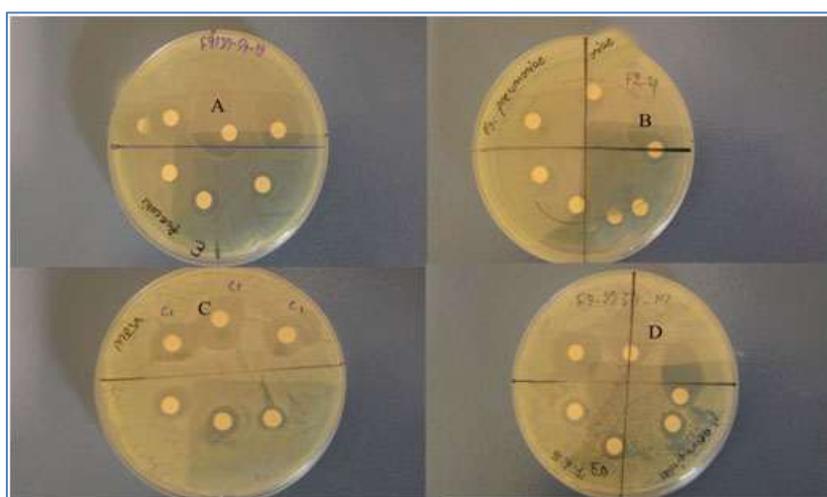
**Table-3: Antibacterial activity of essential oil and organic extracts of *Clerodendrum infortunatum* L**

Bacteria	Zone of growth inhibition in mm						
	Extracts <sup>a</sup>				Standard antibiotics		
	Chloroform	EtOAc	MeOH	EO	TE	ST	ER
EC	14 ± 0.6	15 ± 0.5	18 ± 0.5	22 ± 0.5	24 ± 0.7	16 ± 0.5	13 ± 0.9
MRSA	13 ± 0.5	12 ± 0.7	15 ± 0.4	16 ± 0.4	29 ± 1.1	23 ± 0.5	25 ± 0.7
KP	19 ± 1.1	18 ± 1.2	23 ± 1.2	24 ± 1.2	13 ± 0.5	16 ± 0.9	10 ± 0.5
PA	17 ± 0.7	17 ± 0.5	23 ± 0.5	21 ± 0.5	32 ± 1.5	32 ± 1.2	25 ± 1.2
EF	19 ± 0.6	18 ± 0.8	21 ± 0.7	22 ± 0.7	12 ± 0.6	18 ± 1.1	12 ± 0.5

Values are given as mean ± S.D of triplicate experiment.

<sup>a</sup>Diameter of inhibition zones (mm) of the essential oil and organic extracts around the discs (6 mm) impregnated with 10 µL disc<sup>-1</sup> correspond to 50 µg disc<sup>-1</sup>. Standard antibiotics: TE, Tetracycline (30 µg disc<sup>-1</sup>);

ST, streptomycin (30 µg disc<sup>-1</sup>); ER, erythromycin (15 µg disc<sup>-1</sup>). Bacteria: EC, *Escherichia coli* ATCC 35218; MRSA, Methicillin-resistant *Staphylococcus aureus* (isolate); KP, *Klebsiella pneumoniae* ATCC 700603; PA, *Pseudomonas aeruginosa* ATCC 27853; EF, *Enterococcus faecalis* ATCC 291212.



**Fig-5: Diameter of inhibition zones (mm) of (50 µg disc<sup>-1</sup>): A) Chloroform; B) EtOAc; C) Essential Oil; D) MeOH**

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