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Antibacterial Screening and Antioxidant and Free Radical Scavenging Activity of *Ecballium elaterium*

[1]*Saleh Mosbah Muftah,* [2]*Saleh H. Almajberi and* [3]*Osma S. Mohamed,* [4]*Marei A. Altayar,*
[5]*Muftah A. Nasib,* [5]*Idress Hamad Attitalla,* [5]*Ilham O.A. Al-Hejazi and* [1]*Maraia F. Elmhdwi*

[1]Department of Chemistry (Biochemistry), Faculty of Science, Benghazi University, Benghazi, Libya
[2]Department of Botany (Microbiology), University of Benghazi, Libya
[3]Department of Microbiology, Libyan Academy, Benghazi, Libya
[4]Department of Medical Microbiology and Parasitology,
Faculty of Medicine, Benghazi University, Benghazi, Libya
[5]Department of Microbiology, Faculty of Medicine, Omar Al-Mukhtar University, Al-Bayda, Libya

Abstract: The study was designed to investigate the antioxidant and antibacterial effects of *Ecballium elaterium* "Fruit juice". The phytochemical analysis of *E. elaterium* "Fruit juice" indicates the presence of Phenols and flavonoids. Total phenolics content and flavonoids were found to be 132.46 µg/ml of pyrogallol and 26 µg/ml of quercetin which equivalent to 100 µl of *E. elaterium* "fruit juice" respectively. The antioxidant activity of *E. elaterium*" fruit juice" ranged from 37% at 100µl to 89% at 500 µl. Where found each 100 µl of fruit juice equivalent to 30 µg/ml of vitamin C. The reducing capacity of the *E. elaterium*" fruit juice" found to be 230.17 µg/ml of ascorbic acid equivalent to 100 µl of *E. elaterium* "fruit juice". The *Ecballium elaterium* "Fruit juice" was screened for antibacterial activities against some pathogens viz., *Escherichia coli, Pseudomonas aeruginosa Staphylococcus aureus, Acinetobacter sp. and Klebsiella pneumonia.*The *Ecballium elaterium* "Fruit juice" extracted was found to produce significant inhibition against all the pathogens. Finally, the present study suggests that "Fruit juice"extracted from *Ecballium elaterium* have potential activity as source of natural antioxidant.

Key words: *Ecballium elaterium* · Phytochemical · Antibacterial · Antioxidant

INTRODUCTION

Humans have frequently used plants to treat common infectious diseases and some of these traditional medicines are still part of the habitual treatment of various maladies. It has been reported that 115 articles were published on the antimicrobial activity of medicinal plants in Pub Med during the period between 1966 – 1994, but in the following decade, between 1995 and 2004, 307 were published (Rios and Recio 2005). In a previous study, we have reported the antibacterial effect of extracts against pathogenic bacteria strains (Zeybek *et al*. 2006). The present study describes the evaluation of the antibacterial potency and antioxidant activity of ecballium eletarium [1].

Infectious disease still represent an important cause of morbidity and mortality among humans, especially in developing countries. In recent years one of the more alarming trends in clinical microbiology has been the increasing incidence of resistance to antimicrobial agents among pathogens cause Medicinal plants have been important sources of products in treating common infections and overcoming the problems of resistance and side effects of the currently available antimicrobial agents in the developing countries.

One therapeutic strategy employed to overcome these resistance is the use of combination of drugs, such as B-lactamase inhibitors together with B-lactams [2]. Concurrent administration of two or more drugs is often essential and sometimes mandatory in order to achieve desired therapeutic goal or to treat co-existing diseases [3].

In recent years, the use of traditional medicine has spread in the world and has grown in popularity, not only the populations of developing countries have access, but also those countries where biomedicine occupies an important place in health systems. Natural substances

Corresponding Authors: Muftah A. Nasib, Department of Microbiology, Faculty of Medicine,
Omar Al-Mukhtar University, P.O. Box: 919, Al-Bayda, Libya.

from plants have multiple interests utilized in several industries (food, cosmetics ...). Occupying a prominent place in the group of polyphenols, flavonoids are ubiquitous secondary metabolites in plants. These compounds are known for their numerous biological activities, such as antiviral, anti-inflammatory and anticancer [4].

These numerous properties related to health, well described in epidemiological studies, mainly based on their antioxidant activities [5].

Recent work aimed to isolate new substances from plants and finding other ways of applications in different fields. For these reasons, we are interested in studying the antioxidant activities of polyphenols and flavonoids isolated from different organic fractions of *Ecballium elaterium* (L.). The few studies conducted on this plant, encouraged us to study the antioxidant properties of this plant to enrich knowledge on the biological activities of this plant.

E. elaterium (L.) (Cucurbitaceae) is a medicinal plant found abundantly in the wild South-West Europe and North Africa in stony ground, in the rubble and slope [6]. The plant is known as grass officinal herb and has a long tradition of uses in the Mediterranean basin. It is often used in dropsy (edema), especially pulmonary edema and also as a revulsive in brain diseases [4]. The fresh raw juice is frequently used in the treatment of sinusitis and jaundice by nasal aspirates [5].

Many biological activities of this species have been attributed to cucurbitacins and their glycosylated derivatives such as anti-proliferative activity on various types of cancer cells [6-7]. But his most interesting potential activity can be antiviral [8].

It is logical to consider antioxidants as primary candidates to counteract such toxic effect, accumulating evidence supported the protective effects of antioxidants from medicinal plants against oxidative stress mediated disorders. Studies are going on throughout the world for the search of protective molecules that would provide maximum protection of the liver, kidney as well as other organs and practically very little or no side effects would be exerted during their function in the body [9].

Fruits and vegetables are the major sources of dietary antioxidant vitamins, such as vitamin C, vitamin E and phenolic compounds which are also antioxidant and are numerous and widely distributed in the plant kingdom [10]. Phenolic compounds are widely distributed in the plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging, anti-inflammatory and anti-carcinogenic [11]. Phenolic constituents, such as flavonoids and phenolic acids are especially worthy of notice due to their high anti-oxidative activity [12].

The antioxidants are known to play an important role in protection against disorders which caused by oxidant damage [13]. Antioxidants refer to compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions, they act in one or more of the following ways: as reducing agents, by free radical scavenging and as quenchers of singlet oxygen[14].

The few studies conducted on *Ecballium elaterium*, encouraged us to study the antioxidant and antibacterial effects of this plant to enrich knowledge on the biological activities of this plant. For these reasons, we are interested in studying the antioxidant and antibacterial effects of polyphenols and flavonoids content of *Ecballium elaterium* "Fruit juice"

MATERIALS AND METHODS

Materials

Ecballium Elaterium "Fruit Juice": The ripe fruits of Ecballium elaterium were collected from green mountain area in Benghazi, Libya during November 2013.

Chemicals: 1, 1-Diphenylpicrylhydrazyl radical (DPPHÿ) and silymarin were obtained from Sigma Chemicals, ascorbic acid, Folin-Ciocalteu reagent, ferric chloride, potassium ferricyanide, monobasic dihydrogen phosphate, dibasic monohydrogen phosphate, trichloro acetic acid, sodium carbonate, quercetin and pyrogallol obtained from biochemistry laboratory of chemistry department.

Bacterial Used: Refrence straian of gram-positive and gram-negative bacteria, were obtained from Laboratory of Department of Botany, Faculty of Science, Benhgazi University. which know as multi drag resistant bacteria. Thebacteria used were *Escherichia coli* (MDR)ATCC, *Staphylococcus aureus (MDR) ATCC, Pseudomonas aeruginosa(MDR) ATCC*, Klebsiella pneumonia (MDR) and Acinetobacter sp (MDR).

Methods:

Preparation of Ecballium Elaterium " Fruit Juice": The fruits of Ecballium elaterium are well crushed. The obtained crude juice were refined by filtration using Whatman filter paper No.4 the refined crude juice is used in the estimation of antioxidant activity and antibacterial.

Plant Extract: The fruits were homogenized and then dried in incubator at 37°C. Exposure to light was avoided to prevent the loss of effective ingredients. Approximately 30-40 g of dried material were mixed thoroughly with magnetic stirrer in 200 mL of 80% ethanol at room temperature and the mixture left for 24 h. The insoluble materials were removed by centrifugation at 10 000 rpm for 10 min at 4°C. Then, the extract was evaporated to dryness at 37°C. The extract was weighed and dissolved in sterile distilled water at a concentration of 200 mg/mL and stored at 4°C for assay [15].

Extraction of Cucurbitacine from Fruit Juice: The fruit juice 2 L from fresh fruit 21Kg was partitioned with diethyl ether, ratio 1:1. The organic phase was concentrated under reduced. Solvent was removed at 40-50 0C by rotary evaporator. The yields of extract were recorded [16].

Antioxidant Activities Assays and Quantitative Analysis: All of these experimental have been conducted in biochemistry laboratory at Benghazi University.

Total Phenolic Content (TPC): Total concentration of phenolic compound in the essential oils obtained from Rosmarinus officinalis was estimated using the colorimetric method based on Folin-Ciocalteu reagent [17]. Quantification was done with respect to standerd calibration curve of Pyrogallol the results were expressed as pyrogallol "µg/ml".

Total Flavonoids Content (TFC): Aluminum chloride colorimetric method was used for determination [18]. The calibration curve was obtained by preparing different quercetin solutions in methanol at concentrations "100 to 500 µg/ml".

Reducing Power Assay (RPA): The reducing power was determined according to the[19]. Quantification was done with respect to stander calibration curve of ascorbic acid the results were expressed as ascorbic acid "µg/ml".

Potassium ferricyanide + ferric chloride antioxidant potassium ferricyanide + ferrous chloride.

DPPH Free Radical Scavenging Activity (RSA): The antioxidant activity of the essential oils was measured in terms of hydrogen donating or radical-scavenging ability using the stable DPPH• method as modified by [20]. Radical scavenging activity was expressed as percent of inhibition and was calculated using the formula:

%DPPH "RSA" = [Abs. of Control – Abs. of Sample / Abs. of Control] x 100

Antibacterial Activities of Fruit Juice and Cucurbitacin Extract: Three different concentration of extract juice(100mg/ml.150mg/ml and 200mg/ml and two for cucurbitacin extract(100mg/ml and 200mg/ml were examined for it is antibacterial activity, this method depends upon diffusion from the wells through the solidified agar layer of petri dish to such an extent that growth of the streaked bacteria are prevented entirely, in circular area or inhibition zone around the wells containing the different extract under study, the plates were incubated at 37°C for 18-24 hr. The antibacterial assay plates were incubated at 37°C for 24hr. The effect of fixed and volatile oils on the tested bacteria was compared with the sensitivity of the same bacteria to five antibiotics Colisti sulphate, Amicacin, Amoxycillin, gentamicin and sulphamethoxazole trimethoprim (60µg/ml) [21-23].

RESULTS AND DISCUSSION

Antioxidant Evaluation of the Ecballium Elaterium "Fruit Juice" and Cucurbitacin: The antioxidant activities of the Ecballium elaterium "fruit juice" and cucurbitacin are evaluated by:

Total Phenolic Content (TPC): Results obtained in Table (1) referred to total phenolic content of Ecballium elaterium "fruit juice" where compared with pyrogallol as a standard phenolic compound were each 100 µl of Ecballium elaterium equivalent to 123.46 µg/ml. Phenols are very important plants constituents with multiple biological functions, including antioxidant activity because of their radical scavenging ability due to their OH groups [24].

Total Flavonoids Content (TFC): Total flavonoids content also determined in fruit juice of Ecballium elaterium were compared with quercetin and as illustrated in Table (2) the flavonoid content in fruit juice was 26 µg/ml of quercetin in each 100 µl. Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action. Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity and their effects on human nutrition and health are considerable. According to its action through scavenging or chelating process [25].

Table (1): Total phenolic content (TPC) of *Ecballium elaterium* "fruit juice" compared to pyrogallol according to absorbance

Volume of Ecballium elaterium "µl"	Mean ± Standard Deviation	Concentration of Pyrogallol "µg/ml"	Mean ± Standard Deviation
100	0.525 ± 0.0162	100	0.438 ±0.020
200	0.725 ± 0.020	200	0.725 ± 0.050
300	0.922 ± 0.022	300	1.070 ±0.087
400	1.154 ± 0.036	400	1.307 ±0.027
500	1.361 ± 0.037	500	1.564 ±0.075

Table 2: Total flavonoid content of *Ecballium elaterium* "fruit juice" compared to quercetin according to absorbance.

Volume of Ecballium elaterium "µl"	Mean ± Standard Deviation	Concentration of quercetin "µg/ml"	Mean ± Standard Deviation
100	0.0845 ± 0.055	100	0.307±0.025
200	0.135 ±0.045	200	0.587 ±0.075
300	0.191±0.075	300	0.974 ±0.074
400	0.223 ±0.071	400	1.203 ±0.056
500	0.266 ±0.085	500	1.511 ±0.026

Table 3: Reducing power assay of *Ecballium elaterium* "fruit juice" compared to vitamin C. according to absorbance

Volume of Ecballium elaterium "µl"	Mean ± Standard Deviation	Concentration of vitamin C "µg/ml	Mean ± Standard Deviation
100	0.503 ±0.0448	100	0.201 ±0.0168
200	0.875±0.0965	200	0.495 ±0.0264
300	1.293 ±0.0471	300	0.697 ±0.0308
400	1.563 ±0.0266	400	0.992 ±0.0173
500	2.039 ±0.0401	500	1.201 ±0.0264

Table 4: DPPH radical scavenging of *Ecballium elaterium* "fruit juice" compared to vitaminC according to % inhibition.

Volume of Ecballium elaterium "µl"	Percent of inhibition %	Concentration of vitamin C "µg/ml	Percent of inhibition %
100	37%	100	44%
200	49%	200	54%
300	60%	300	67%
400	73%	400	76%
500	89%	500	84%

Table 5: Arithmetic mean values ± S.D of Phytochemical screening of *Ecballium elaterium* "fruit juice" according to absorbance

Ecballium elaterium	Total phenolic content	Total flavonoid content	Reducing power	DPPH• radical
100 µl	0.525 ± 0.0162	0.0845 ± 0.055	0.503 ±0.0448	1.778±0.059
200 µl	0.725 ± 0.020	0.135 ±0.045	0.875±0.0965	1.478± 0.092
300 µl	0.922 ± 0.022	0.191±0.075	1.293 ±0.0471	1.121±0.073
400 µl	1.154 ± 0.036	0.223 ±0.071	1.563 ±0.0266	0.447±0.088
500 µl	1.361 ± 0.037	0.266 ±0.085	2.039 ±0.0401	0.427±0.106

Reducing Power Assay (RPA): The reducing capacity of Ecballium elaterium "fruit juice" has been compared with the ascorbic acid according to the results that mentioned in Table (3) were notice each 100 µl of Ecballium elaterium "fruit juice" equivalent to 30 µg/ml. The reducing capacity of cucrbitacine has been compared with the ascorbic acid according to the results that mentioned in Table (6) were notice each 100 µl of cucrbitacin equivalent to 30 µg/ml. *E. elaterium* might contain higher amount of reducers, which could react with free radicals to stabilize and block radical chain reactions [25].

The DPPH• Radical Scavenging Activity: The results of the DPPH• radical scavenging activity of Ecballium elaterium "fruit juice" are shown in Table (4). These results are compared with the well-known antioxidant ascorbic acid the percent of inhibition started from 37% at 100 µl to 89% at 500 µl and recorded from 44% at 100 µg/ml to 84% at 500 µg/ml in ascorbic acid. The results of the DPPH• radical scavenging activity of cucrbitacine are shown in Table (7). The role of antioxidant is its interaction with oxidative free radicals. The essence of DPPH assay is that the antioxidant reacts with the stable free radical 1, 1-diphenyl-2-picrylhydrazyl (deep violet color) and converts it to 1, 1-diphenyl-2-picrylhydrazine with a yellow color. The degree of discoloration indicates the scavenging potential of the sample as an antioxidant [26].

Table 6: Reducing power assay of *cucurbitacin*" compared to vitamin C according to absorbance

Volume of cucrbitacin "µl"	Mean ± Standard Deviation	Concentration of vitamin C "µg/ml	Mean ± Standard Deviation
100	0.303±0.05	100	0.201 ±0.0168
200	0.646±0.032	200	0.495 ±0.0264
300	0.989±0.017	300	0.697 ±0.0308
400	1.474±0.036	400	0.992 ±0.0173
500	2.050±0.022	500	1.201 ±0.0264

Table 7: DPPH radical scavenging of *cucrbitacine* compared to vitamin C according to % inhibition

Volume of cucrbitacin "µl"	Percent of inhibition %	Concentration of vitamin C "µg/ml	Percent of inhibition %
100	60%	100	44%
200	74%	200	54%
300	85%	300	67%
400	89%	400	76%
500	93%	500	84%

Table 8: Effect of ethanol extract from *Ecballium elaterium* "fruit juice"as antibacterial and ethanol as control

	Zone of inhibition in (mm± standard deviation)				
Ethanol extract from *Ecballium elaterium* "fruit juice"	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas aereuginosa*	*Klebsiella pneumonia*	*Acinetobacter* sp
100mg/ml	0±0.00	11±0.05	16±0.02	16±0.07	15±0.04
150mg/ml	0±0.00	11±0.01	21±0.09	18±0.02	18±0.02
200mg/ml	0±0.00	11±0.03	24±0.01	20±0.02	21±0.01
Control	0±0.00	0±0.00	11±0.01	10±0.01	0±0.00

Each assay in these experiments was repeated three times and the results (mm of zone of inhibition) were expressed as average values (± standard deviation). Mean inhibition zone diameter (mm)after 24 h of incubation.

Table 9: Effect of *cucrbitacine* as antibacterial and methanol as control.

	Zone of inhibition in (mm± standard deviation)				
Cucrbitacine	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas aereuginosa*	*Klebsiella pneumonia*	*Acinetobacter sp*
100mg/ml	0±0.00	0±0.00	21±0.02	17±0.02	19±0.01
200mg/ml	0±0.00	21±0.04	26±0.01	19±0.07	21±0.03
Control	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00

Each assay in these experiments was repeated three times and the results (mm of zone of inhibition) were expressed as average values (± standard deviation). Mean inhibition zone diameter (mm)after 24 h of incubation

Table 10: Antibiotic activity of different type of bacteria

	Antibiotic				
Bacteria	Colisti sulphate	Amicacin	Amoxycillin	Gentamycin	Sulphmethoxazole
Escherichia coli	0±0.00	15±0.02	0±0.00	0±1	0±0.00
Staphylococcus aureus	2±0.01	13±0.02	3±0.01	6±0.01	19±0.03
Pseudomonas. aeruginosa	3±0.01	9±0.01	0±0.00	5±0.02	0±0.00
Acinetobacter sp	6±0.03	0±0.00	0±0.00	0±0.00	0±0.00
Klebsiella pneumonia	4±0.01	12±0.04	2±0.01	1±0.01	0±0.00

Each assay in these experiments was repeated three times and the results (mm of zone of inhibition) were expressed as average values (± standard deviation). Mean inhibition zone diameter (mm)after 24 h of incubation

Antibacterial Evaluation of the Ethanol Extract from *Ecballium elaterium* "Fruit Juice" and Cucurbitacin: From the various available methods of testing antibacterial activity, the Hole-plate diffusion method, measuring inhibition zone, were used for detection of sensitivity of given organisms, an These methods gives optimum growth conditions, for all organisms and avoid the problem of sterilization plant extracts prior to testing.

The inhibitory effect of different concentration of ethanol extract from *Ecballium elaterium* "fruit juice" are show in the Table (8). Diammter of inhibition zone were measured in millimeter at 18 – 24 hr. most activities are shown from ethanol extract from *Ecballium elaterium* "fruit juice" against *Klebsiella pneumonia* (Fig. 2), *Pseudomonas aereuginosa* (Fig. 3), *Acinetobacter sp* (Fig. 4), *Staphylococcus aureus* (Fig. 5), with zone of

Fruit (21 Kg)
↓
Fruit juice (2 L)
↓
Extracted with diethyl ether 1:1
↓ rotary evaporator
Viscous liquid, partitioned with CHCl3:MeOH 9:1
↓
The organic phase
↓ rotary evaporator
Viscous liquid, partitioned with CHCl3:MeOH 9:1
↓ partation with CHCl3
The organic phase
↓
Filtrate
↓ rotary evaporator
Viscous liquid, partitioned with CHCl3:MeOH 9:1
↓ dissolved in ethyl acetate
Cucurbitacin

Fig. 1: Extraction of cucurbitacin

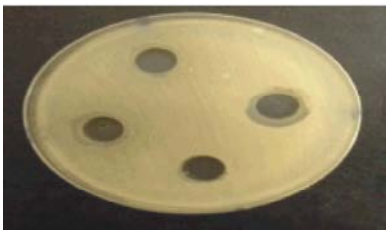

Fig. 2: Effect of ethanol extract from *Ecballium elaterium* "fruit juice"aganist *Klebsiella pneumonia*

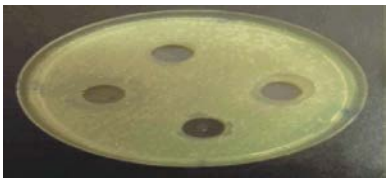

Fig. 3: Effect of ethanol extract from *Ecballium elaterium* "fruit juice"aganist *Pseudomonas aereuginosa*

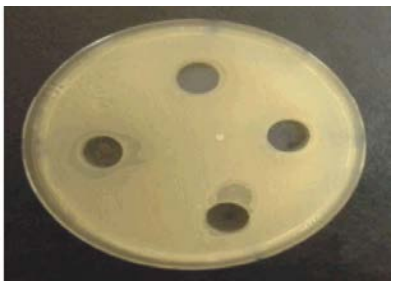

Fig. 4: Effect of ethanol extract from *Ecballium elaterium* "fruit juice"against *Acinetobacter sp.*

Fig. 5: Effect of ethanol extract from *Ecballium elaterium* "fruit juice"against *Staphylococcus aureus*

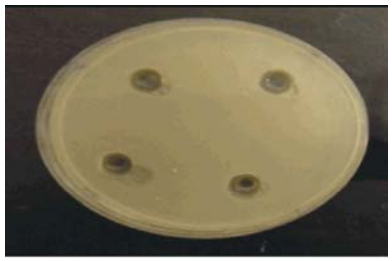

Fig. 6: Effect ethanol extract from *Ecballium elaterium* "fruit juice"aganist *Escherichia coli.*

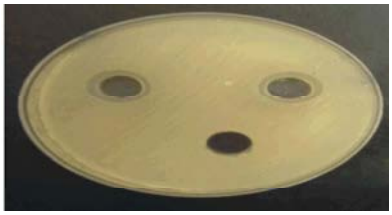

Fig. 7: Effect of cucurbitacin aganist *Klebsiela pneumonia*

Fig. 8: Effect of cucurbitacin aganist *Pseudomonas aereuginosa*

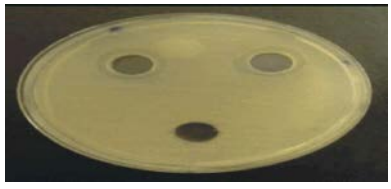

Fig. 9: Effect of cucurbitacin against *Acinetobacter sp.*

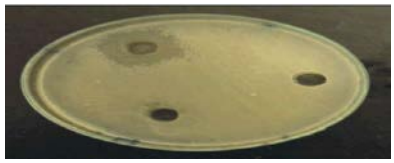

Fig. 10: Effect of cucurbitacin aganist *Staphylococcus aureus*

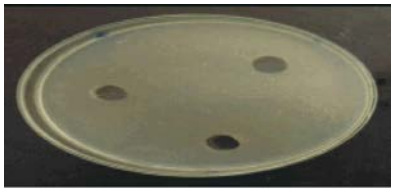

Fig. 11: Effect of cucurbitacin against *Escherichia coli.*

inhibition range from 11 to 16 mm at concentration 100mg/ml, the zone of inhibition range from 11 to 21 at concentration of 150mg/ml and zone range from 11 to 24 at concentration of 200mg/ml. No sensitivity were observed from ethanol extract from *Ecballium elaterium* "fruit juice" aganist *Escherichia coli* (Fig. 6).

The inhibitory effect of different concentration of cucurbitacine are show in the Table (9). Diammter of inhibition zone were measured in millimeter at 18 – 24 hr. most activities are shown from cucurbitacine against *Klebsiella pneumonia* (Fig. 7), *Pseudomonas aereuginosa* (Fig. 8), *Acinetobacter sp* (Fig. 9), *Staphylococcus aureus* (Fig. 10), with zone of inhibition range from 17 to 21 at concentration of 100mg/ml and zone of inhibition range from 19 to 26 at concentration 200mg/ml. No sensitivity were observed from cucurbitacine aganist *Escherichia coli* (Fig. 11).

Antibiotic Activity of Different Type of Bacteria: Antibiotic used in this study for comparison give different effects against bacteria Table (10). This studied that the *Ecballium elaterium* rich in phenolic constituent such as minor components include hydroquinol, 2-nitroquinol, 4-hydroxy acetophenone, 4-hydroxy-3-methoxy acetophenone [27].

Ecballium elaterium is a rich source of flavonoids such as phytomelin, also known as rutin, quercetin-3-rutin with antioxidant effect. The amount of Phytomelin per 1.0 g of dry powder of flowers = 1.59 ± 0.12(mg/mL), fruits = 1.84± 0.13(mg/mL) and leaves = 8.54 ± 0.56(mg/mL) [28].

Flavonoids of plant origin are reported to have potent antioxidants and homeostatic balance between pro-oxidant and anti-oxidants is known to be important for maintenance of health as well as prevention from various degenerative diseases. Flavanoids constitute major group of compounds which act as primary antioxidants and are known to react with hydroxyl radicals, superoxide anion radicals and lipid peroxyradicals, protect DNA from oxidative damage, inhibitory against tumor cell and possess anti-inflammatory and antimicrobial properties [29].

CONCLUSION

In the present study, *Ecballium elaterium* "fruit juice" possessed strong anti-oxidant activity. The anti-bacterial activity of *Ecballium elaterium* "fruit juice" may be due to its free radical-scavenging and antioxidant activity, resulting from the presence of some phenolic compounds in the fruit juice of plant.

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