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FLAVONOID AND PHENOLIC CONTENTS, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF ROOT AND AERIAL PARTS OF *CENTAUREA PUMILIO* L.

Asmaa Gamal*, Amal A. Al-Gendy, Rasha Adel and Samia S. Hafez

Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University, 44519, Egypt.

***Corresponding Author: Asmaa Gamal**

Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University, 44519, Egypt.

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ABSTRACT

The ethanolic extract and its soluble fractions (light petroleum, dichloromethane and ethyl acetate) of *Centaurea pumilio* root and aerial parts were subjected individually to the evaluation of their flavonoid and phenolic contents, antioxidant and antimicrobial activities. Total phenolics and flavonoids contents were quantified by Folin-Ciocalteu and aluminum chloride - potassium acetate colorimetric methods. Tested extracts and fractions were screened for their radical scavenging potential using a 1,1- diphenyl-2-picryl-hydrazyl (DPPH) *in-vitro* model system for antioxidant activity. Additionally, antimicrobial activity was carried out against two Gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), two Gram negative bacteria (*Enterobacter cloacae* and *Escherichia coli*) and antifungal against two fungal strains (*Aspergillus fumigatus* and *Candida albican*) using well diffusion method. Root ethanolic extract exhibited higher phenolic and flavonoid contents than aerial parts. DPPH scavenging activity of root extract was higher than aerial parts. Ethyl acetate soluble fraction of root showed the highest antioxidant activity with SC_{50} = 35.9 ± 0.81 µg/ml followed by methylene chloride fraction with SC_{50} 40.7 ± 0.5 µg/ml compared with ascorbic acid (SC_{50} 14.2 ± 0.5 µg/ml). Additionally, the total ethanolic extract of the aerial parts showed antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* with a percentage inhibition of diameter growth reaches 50% and 43.33%, respectively referred to gentamycin standard. Moreover, ethanolic extract of the root exhibited activity against *Bacillus subtilis* with a percentage inhibition of diameter growth reaches 38.46% compared to the same standard.

KEYWORDS: *Centaurea pumilio*, Asteraceae, Antioxidant, Antimicrobial, Total flavonoid and phenolic contents.

INTRODUCTION

Plants have the ability to synthesize chemical compounds that are used to perform important biological functions.[1] It is well known that the plant flavonoids and phenols in general, are highly effective free radical scavengers and antioxidants. So,the higher the total polyphenolic and flavonoidal content, the greater is the antioxidant activity.[2] In various pathological conditions such as tissue injury and inflammation process, reactive oxygen species (ROS), play a vital function. Scavenger agents like antioxidants will prevent biological systems to be damaged by the free radical damages.[3]

Antimicrobial drugs are effective for the control of bacterial and fungal infections. The frequent use of antibiotics resulted in the emergence of a number of resistant bacterial strains and the antimicrobial compounds from plants may inhibit bacteria by a different mechanism than the antibiotics do and may have clinical value in the treatment of resistant microbial strains.[4]

Natural products from medicinal plants are known to be chemically balanced, effective with lower side effects than synthetic medicines. According to WHO, plant medicines are pivotal agent for the health of about 80% of the world's population, especially developing countries.[5]

The genus *Centaurea* L. is one of the biggest genera in family Asteraceae which includes more than 800 species distributed mostly in Mediterranean region, Europe, Asia, tropical Africa, America and Australia, while in Egypt it is represented by 17 species.[6,7]

According to the literature survey of *Centaurea pumilio* L, it had been found that several compounds including flavonoids, phenolics, sterols, triterpenes, sesquiterpene lactones and coumarins were previously isolated, identified and characterized in this plant[8] or *invitro* propagated tissues.[9] Domestic use of the peeled root of *C. pumilio* in the management of diabetes and bad breath was reported.[10]

The objective of the present study is to extract and fractionate the root and aerial parts of *C. pumilio* in order to determine their phenolic and flavonoid contents and to assess their antioxidant and antimicrobial activities.

MATERIALS AND METHODS

Plant materials

The root and aerial parts of *Centaurea pumilio* L. [Syn. *Aegialophila pumilio* (L. Bioss), *Centaurea pumila* L.] were collected from north coast region, Egypt. The identity of the plant was confirmed by Prof. Abdel-Halim Abdel-Mogaly, herbarium of horticultural research institute, agricultural research center, ministry of agriculture, Dokki, Giza, Egypt and *C. pumilio* voucher specimen No. 4112-CAIM was kept in the institute herbarium.

Extraction and fractionation

The air dried powdered root and aerial parts of *C. pumilio* L. (200 and 250 g, respectively) were separately extracted by cold maceration (5 times) using 80% aqueous ethanol till complete exhaustion. The solvent was distilled off under reduced pressure at 50 °C to give 25 and 30 g, respectively. Each residue was dissolved in methanol: water mixture (1:9) and partitioned against light petroleum, dichloromethane and ethyl acetate consequently. These fractions were dried over anhydrous sodium sulphate and concentrated to yield 2.6 g, 0.5 g and 2.2 g for root and 3 g, 0.5 g and 3 g for aerial parts, respectively.

Quantitative estimation of the total flavonoid and phenolic contents

Total flavonoid content was evaluated using the aluminum chloride-potassium acetate spectrophotometric assay.[11] Crude ethanolic extracts of root and aerial parts were dissolved in 95% ethanol (2 mg/ml). Three replicates were carried out and total flavonoid content was expressed as mg of rutin equivalents/g extract and as mg of quercetin equivalents/g extract by using calibration curve generated with rutin (6.25-200 µg/ml) in 95% ethanol (v/v), and quercetin (3.125-200 µg/ml) in 95% ethanol.

Spectrophotometric determination of the total phenolic content was carried out using the Folin-Ciocalteu colorimetric method.[12] The crude ethanolic extracts of root and aerial parts were dissolved in methanol at a concentration of 2 mg/ml. Three replicates were carried out and total phenolics were expressed as mg of gallic acid equivalents (mg GAE)/g extract deduced from calibration curve of standard gallic acid (40-300 µg/ml).

Antioxidant activity using DPPH free radical scavenging method

The antioxidant activity of the total ethanolic extracts of root and aerial parts in addition to, dichloromethane and ethyl acetate soluble fractions of root of *C. pumilio* were evaluated in accordance to 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay[13] at different concentrations, 10, 20, 40, 80, 160 and 320 ug/ml methanol.

The percentage of DPPH radical scavenging was calculated related to the formula.

% DPPH radical scavenging= [(AC- AT)/ AC x 100],

Where AC is the absorbance of the control and AT is the absorbance of the tested sample in DPPH solution. Relationship between the percentage of DPPH radical-scavenging and concentrations of samples and ascorbic acid standard (µg/ml) were determined to obtain the SC_{50}.

Antimicrobial activity using well diffusion method

Antibacterial and antifungal activities of total ethanolic extracts of root and aerial parts in addition to, light petroleum, dichloromethane and ethyl acetate soluble fractions of the aerial parts of *C. pumilio* were determined using the well diffusion method[14] against two Gram positive bacteria (*Staphylococcus aureus* [RCMB 010010 ATCC 25923] and *Bacillus subtilis* [RCMB 015 NRRL B-543]), two Gram negative bacteria (*Enterobacter cloacae* [RCMB 001 ATCC 23355] and *Escherichia coli* [RCMB 010052 ATCC 25922]) and two fungal strains (*Aspergillus fumigatus* [RCMB 002008] and *Candida albicans* RCMB [005003 ATCC 10231]) obtained from the regional center for mycology and biotechnology Al-Azhar University, Cairo, Egypt. Nutrient agar medium and Saboroud dextrose agar were applied for bacteria and fungi strains, respectively. The tested samples were dissolved in dimethyl sulfoxide (DMSO) at concentration of 10 mg/ml. Positive controls used for assay include, Gentamicin (4 µg/ml) as antibacterial agents and ketoconazole (100 µg/ml) as antifungal. The wells were filled with 100 µl from stock solution of all samples, the standards and DMSO as a negative control. Cultures were incubated at 37ºC for 24 hours for bacteria and for 2-7 days for fungi.

$$\% \text{ of inhibition} = \frac{\text{Inhibition zone diameter of sample - Inhibition zone diameter of solvent}}{\text{Inhibition zone diameter of standard - Inhibition zone diameter of solvent}} \times 100$$

Minimal inhibitory concentration (MIC) was also determined for tested extracts and fractions which showed significant inhibition zone diameters against the tested organisms.

The concentration of tested samples were ranged from 5 to 250 µg/mL according to Washington and Wood.[15] Inocula were obtained from a suspension containing approximately $1\text{-}2\times10^8$ colony-forming unit/mL (cfu/mL). To obtain turbidity comparable to that of the 0.5 McFarland standards, the turbidity of the actively growing broth culture was adjusted with sterile broth.[16]

Statistical analyses

All results of biological activities were expressed using Microsoft Excel (2010) and recorded three times. Each value represents the mean of three readings ± S.D and all bars in the figures represent S.D. The SC_{50} was

determined as the drug concentration required to scavenge 50% of the free radicals.

RESULTS AND DISCUSSION

Quantitative estimation of the total flavonoid and phenolic contents

Total flavonoid content of the ethanolic extracts of *C. pumilio* root and aerial parts were estimated by aluminum chloride potassium acetate spectrophotometric method, and expressed as quercetin and quercetin equivalents by reference to calibration curves where y = 0.001 x - 0.012 and r2 = 0.985 in case of rutin as standard while y = 0.0008 x + 0.0097 and r2 = 0.986 in case of quercetin as standard, **Fig. 1**.

Total phenolic content of ethanolic extracts of *C. pumilio* root and aerial parts were estimated by Folin-Ciocalteu method, are expressed as gallic acid equivalents (GAE) by reference to standard calibration curve y = 0.006x - 0.200, where, $r^2 = 0.988$.

The results showed that ethanolic extract of root showed the highest amount of flavonoids (27.7 ±1.12 and 34.69±1.53 µg/g.DW) expressed as rutin and quercetin equivalents, respectively. Also they showed the highest values of total phenolic (38.70 ±1.02µg/g.DW) expressed as gallic acid equivalent as shown in table (1).

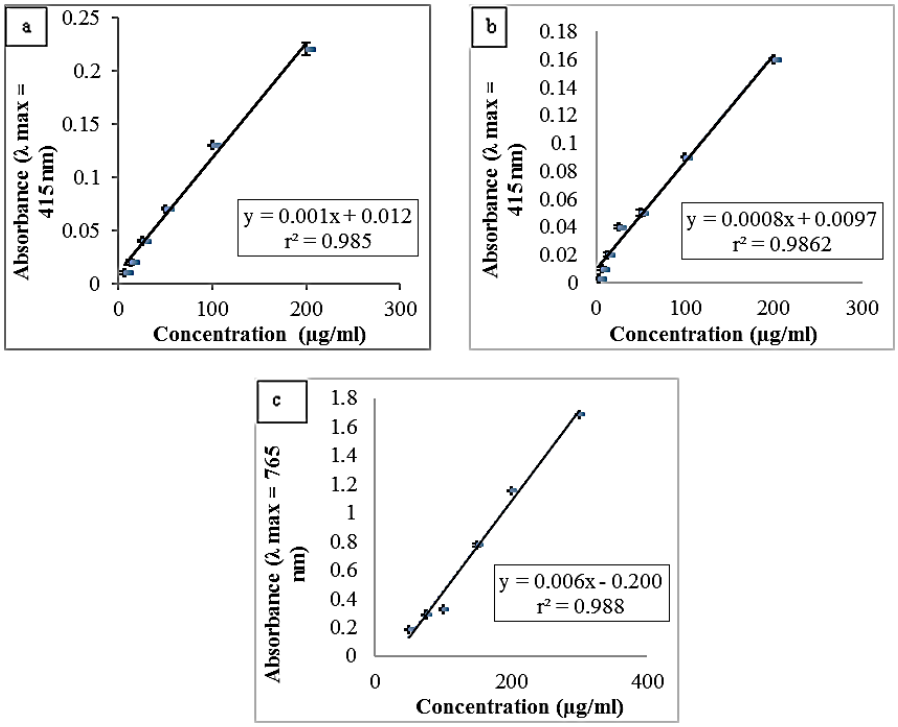

Fig. 1: Calibration curves for standard flavonoids and phenolic compound. (a) Rutin, (b) Quercetin, (c) Gallic acid.

Table (1): The total flavonoid and phenolic contents of the different total ethanolic extracts.

Total ethanolic extracts	Total flavonoids mg rutin/ g extract	Total flavonoids mg quercetin/ g extract	Total phenolic mg GAE/ g extract
Root	27.7	34.69	38.70
Aerial parts	22.28	27.92	29.59

Antioxidant activity using DPPH free radical scavenging method

Phenolic compounds and flavonoids have an important role as primary antioxidants or free radical scavengers. This effect is mainly due to their redox properties that take part in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides. As well as, it has the ability to chelate metals, inhibit lipoxygenase and scavenge free radical.[17]

Results of the antioxidant activity (DPPH scavenging percentage and SC_{50} values) of the total ethanolic extracts and fractions of *C. pumilio* as well as ascorbic acid are shown in **Fig. 2 and 3**. Total ethanolic extract of root exhibited antioxidant activities with SC_{50} = 43.1± 1.2 µg/ml compared to ascorbic acid 14.2 ± 0.5 µg/ml. On the other hand, total ethanolic extract of aerial parts showed antioxidant activities with SC_{50} = 74 ± 0.5 µg/ml. This means that root extract is more effective as antioxidant than aerial parts. Their activity can be attributed to their flavonoid and phenolic contents. So, dichloromethane and ethyl acetate soluble fractions of root part were evaluated for their antioxidant activities to determine the most active fraction. Ethyl acetate soluble fraction exhibited a higher antioxidant activity with SC_{50} = 35.9 ± 0.81 µg/ml than dichloromethane soluble

fraction with SC_{50} 40.7 ± 0.5 µg/ml. It is well known that the plant flavonoids and phenols acted as free radical scavengers and antioxidants. So, the higher the total polyphenolic and flavonoidal content, the greater is the antioxidant activity.[2]

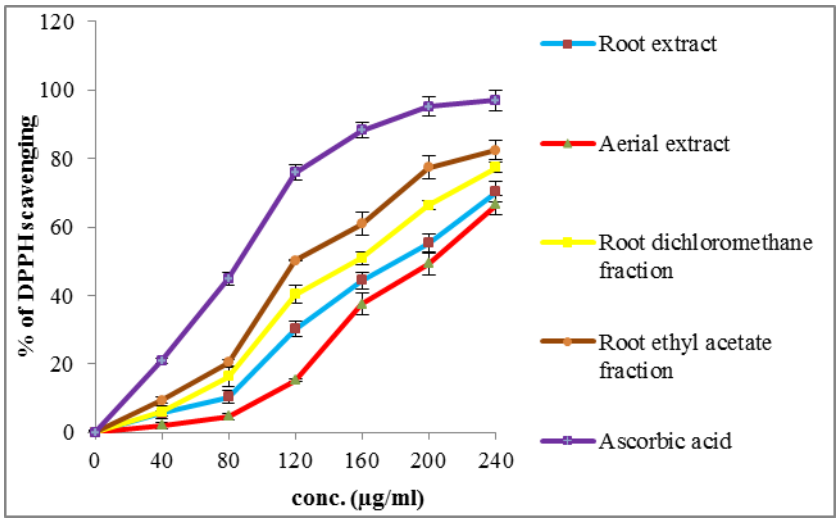

Fig. 2: DPPH scavenging percentage of total ethanolic extracts of root, aerial parts, root dichloromethane and ethyl acetate soluble fractions compared to standard ascorbic acid.

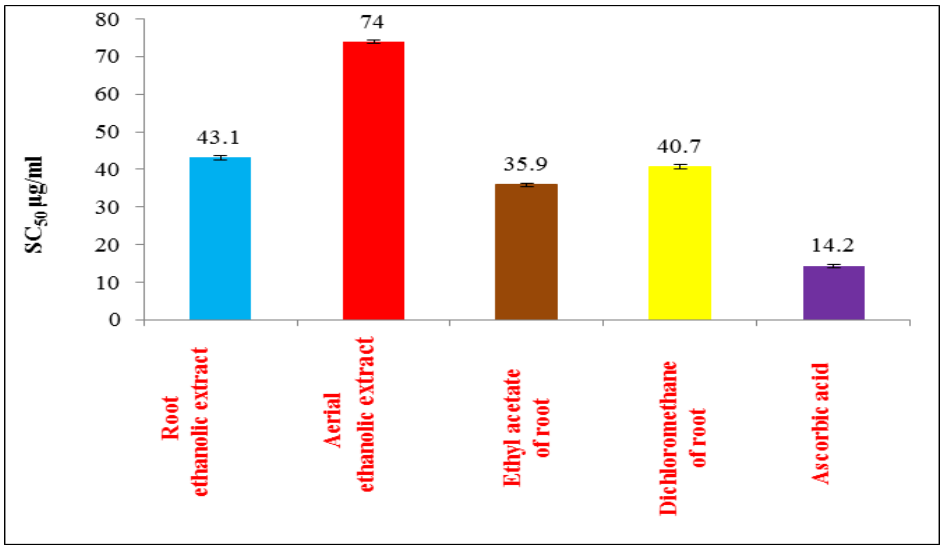

Fig. 3: SC_{50} (µg/ml) of different extracts and fractions of *C. pumilio* compared to standard ascorbic acid.

Antimicrobial activity

Antibacterial and antifungal activities of total ethanolic extracts of root and aerial parts and different soluble fractions against different microorganisms by well diffusion technique were expressed as diameter of inhibition zone, percentage of activity and MIC as shown in tables (2 and 3) and **Fig. 4 and 5.**

The total ethanolic extract of aerial parts showed antibacterial activity against *S. aureus* and *E. coli* with a percentage inhibition of zone diameter growth reaches 50% with MIC 2.5 mg/ml and 43.33% with MIC 1.25 mg/ml, respectively. Additionally, ethanolic extract of root showed activity against *Bacillus subtilis* with a percentage inhibition of zone diameter growth reaches 38.46% with MIC 5 mg/ml. No antifungal activity for both extracts was observed against *C. albicans* or *A. fumigatus.*

In accordance with results, extract showed the highest percentage inhibition of diameter growth, its different solvent soluble fractions, light petroleum, dichloromethane and ethyl acetate, were tested against *S. aureus and E. coli*. Dichloromethane soluble fraction exhibited effective antibacterial activity against *S. aureus* with a percentage inhibition of diameter growth reaches 50% with MIC 1.25 mg/ml and light petroleum soluble fraction showed the highest anti bacterial activity against *E. coli* with a percentage inhibition of diameter growth reaches 66.66% with MIC 0.15 mg/ml.

Table (2): Antimicrobial activity of the total ethanolic extract of root and aerial parts of *C. pumilio.*

Tested sample	Inhibition zone diameter (mm ±S.D) (% of inhibition)					
	Gram +ve bacteria		Gram -ve bacteria		Fungi	
	B. subtilis	***S. aureus***	***E. coli***	***E. cloacae***	***C. albicans***	***A. fumigates***
Root ethanolic extract	10 ± 0.44 (38.46%)	NA*	NA*	NA*	NA*	NA*
Aerial parts ethanolic extract	NA*	12 ± 0.63 (50%)	13 ± 0.24 (43.33%)	NA*	NA*	NA*
Standard						
Gentamicin	26± 0.16 (100)	24± 0.58 (100)	30± 0.52 (100)	30± 0.25 (100)	NA*	NA*
Ketoconazole	NA*	NA*	NA*	NA*	20± 0.44 (100)	17± 0.32 (100)

NA*: No activity

Table (3): Antimicrobial activity of light petroleum, dichloromethane and ethyl acetate soluble fractions of aerial part of *C. pumilio*.

Tested sample	Inhibition zone diameter (mm ±S.D) (% of inhibition)	
	Gram +ve bacteria	Gram -ve bacteria
	S. aureus	***E. coli***
Light Petroleum fraction	10 ± 0.25 (41.66%)	20 ± 0.44 (66.66%)
Dichloromethane fraction	12 ± 0.32 (50%)	15 ± 0.63 (50%)
Ethyl acetate fraction	8 ± 0.37 (33.33%)	18± 0.25 (60%)
Standard		
Gentamicin	24 ± 0.58 (100%)	30± 0.52 (100%)

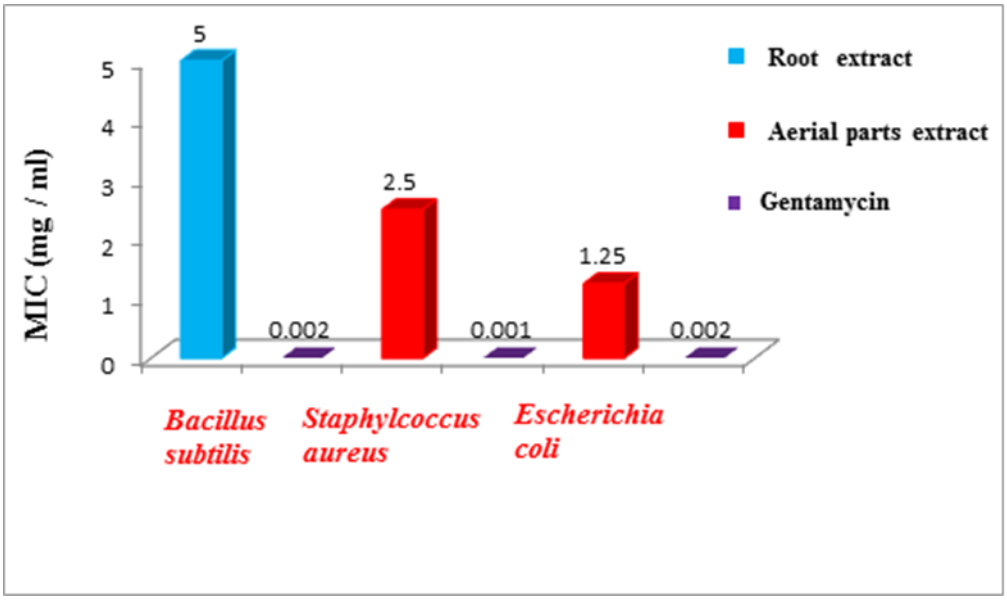

Fig. 4: Minimum inhibitory concentrations (MIC) of the total ethanolic extract of *C. pumilio* root against *Bacillus subtilis* and the total ethanolic extract of aerial parts against *Staphylococcus aureus* and *Escherichia coli* . Gentamicin was used as a positive control.

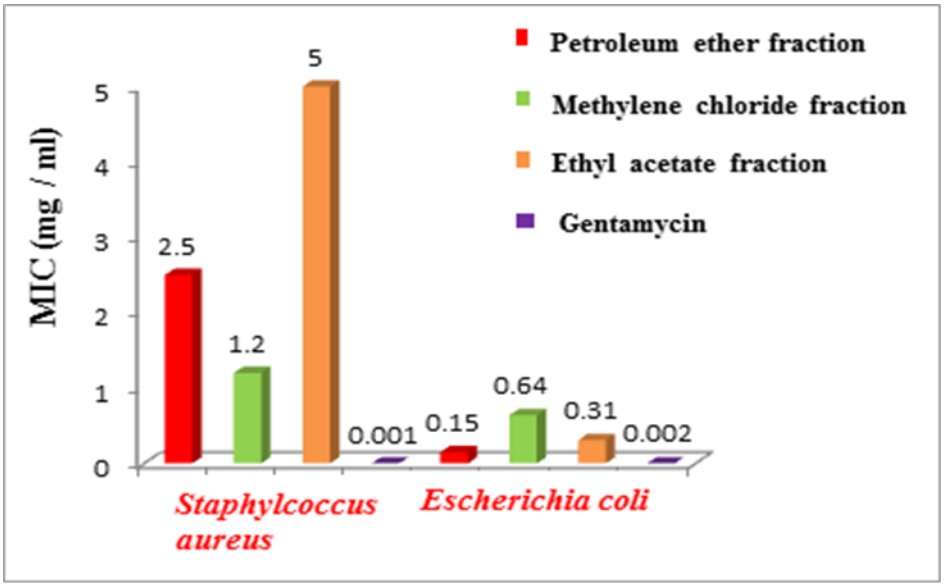

Fig. 5: Minimum inhibitory concentrations of the light petroleum, methylene chloride and ethyl acetate soluble fractions of aerial parts of *C. pumilio* against *Staphylococcus aureus* and *Escherichia coli*. Gentamicin was used as a positive control.

CONCLUSION

The current study is considered the first attempt to extract and assess the antioxidant and antimicrobial activities of *Centaurea pumilio* L root. Significant antioxidant activity of the root ethanolic extract may be attributed to its the high flavonoid and phenolic contents detected in extract. The highest antimicrobial activity was achieved by aerial parts extract against *S. aureus* and *E. coli*.

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