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Biological Activity of *Calligonum comosum* Extracts as Antibacterial and Antioxidant

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The regional geography of Saudi Arabia supports the plant diversity of global, regional and local medicinal plants.There are hundreds of plant species that are of valuable in folk medicine, most of these plants is still under study or they did not yet examinedas a medical value, the medicinal plants in Kingdom are rare and still under the danger of extinction because of rapid change in both environment and development activities, *Calligonum comosum* (stem and fruits) is selected to be studied through this research, in terms of chemical analysis and the impact of plant extractions that undergoes sequential separation using ethanol, chloroform, petroleum ether, ethyl acetate and n-butanol solventsto be used as antioxidant and antibacterial extracts. The processes of extraction, separation and identification of the *Calligonum comosum* compounds were carried out using GC-MS instrument. The effect of plant extracts as antioxidation was of greatest impact for the stem extract in the inhibition of free radicals, where the percentage of inhibition was 74.303% with the concentration of 8mg/ml, followed by the effect of the fruit extract where inhibition ratio reaches 65.74% at the same concentration, followed by the roots extract with inhibition ratio of 17.9%. The minimum inhibitory concentration MIC of each extract showed a recognizable effect on the studied bacterial strains. The stem extract with chloroform and Ethyl acetate solvents has a better effect on the bacterial strain *Bacillus subtilis* and achieved lowest inhibitory concentration, and for the fruit extract, the solvent n-butanol gives the best effect by showing low inhibitory concentration of the bacterial strains *Escherichia coli*, *Shigella sonnei* and *Bacillus subtilis*.

Key words: *Calligonum comosum*; Antibacterial; Antioxidant.

At the present time medicinal plants occupies a great position in the industrial production, and is one of the main sources of prescription drugs or a source of the materials used in the preparation of the drug in form of extracts or active ingredients. Because of the problems related to the use of antibiotics such as penicillin resistance and the side effects of using chemical drugs that shown by scientific research by the mean of harmful side effects of some medications manufactured either because of our lack of knowledge or because they are made of concentrated chemicals that being prepared in the laboratory under harsh conditions of chemical reactions (Bhuraneswari *et al*., 2002).

As the interest in medicinal plants increased, we chose to study calligonum plant because of its economic, environmental and medical importance. Riadh *et al*. 2011; make organic studies on the *Calligonum comosum* found and they found that the plant extracts are useful in alleviating

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significantly the food contamination byListeria bacterium. Badria *et al*., 2007; studied *Calligonum comosum* plant in terms of the effect of the plant extracts on mice and shrimp;and they noticed the effectiveness of these extracts as anti-inflammatory and anti-stomach ulcer, and discouraging the growth of cancer cells.

Ackacha, 2010; studied the stem of *Calligonum comosum* and found that the plant absorbed some heavy elements like zinc and reduces significantly its presence in the aqueous solution.

El-Hawary and Kholief 1990, studied the effect of extracts from some *polygonaceae* plant on rats in terms of blood sugar and they concluded that these extracts are of effective impact on reducing blood sugar of the experimental rats.

This research aims to extract the plant chemical content using different solvents and to identify these chemical compounds in addition to study the effect of these extracts as antioxidant and antibacterial.

MATERIALS AND METHODS

Samples collection

The samples from the stem and fruits of *Calligonum comosum* were collected from Thumama area, Riyadh, in spring season of 2011.

100 gm of the dry sample were weighted and placed on 1000ml flask, 200ml of ethanol 95% conc. were added (Nwosu okafor, 1995) followed by evaporation to precipitate the effective materials as a solid matter. The solid matter were dissolved using a mix of ethanol and water 40% and 60% respectively. Sequential separation is made using alcoholic solvents as petroleum ether; chloroform; ethyl acetate; and n-butanol are used respectively according to Grand *et al*. (1988).

Estimating the antioxidant effectiveness

The effectiveness of the ant-oxidant extracts were estimated using partial anti free radicals method DPPH (Molyneux 2004) that depends on the inhibition of free radicals where it leaves for 30 minutes directly with the extracts to interacts with the partial anti free radical DPPH, that turns into DPPH-H with more stable free radical as it losses adsorption of the maximum wave »max = 517 nm length. The calculation of the inhibition percentage from the relationship I% = (A0 - Ai) / A0 × 100 (Roy *et al*. 2010) where: A0 is optical absorption coefficient of the free radical in the absence of the extracts,and Ai is the optical absorption coefficient of the mixture (free radical + extracts) after 30 minutes.

The effect of plant extracts on bacterial strains

The experiment was conducted on five pathogenicbacterial strains *Bacillus subtilis* (ATCC 10400), *Escherichia coli* (ATCC 442), *Shigella sonnei* (ATCC 11060), *Pseudomonas aureus* (ATCC 27853) and *Staphylococcus aureus* (ATCC 29213). Tablets of Five filter papers with 6mm diameter are prepared and placed in each dish the upper one was carryingthe solvent and the remaining four carried the plant extracts with concentration of 0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml, and 0.8 mg/ml with amount equal 50 microns per each tablet.The environmental dish prepared for the development of bacteria is pollination by 150 microns of the environmentalbacterial with concentration of 1×10^{16} cell bacteria/mlincubated at a temperature of 37°C for 24 hours, according to the discs test method (Desk diffusion test) and then the results of incubation are monitored.

Statistical Analysis

Statistical analysis was carried out to identify the significant values of the obtained results using SPSS V19 program.

Determining the minimum inhibitory concentrations values of plant extracts (MIC)

The minimum inhibitory concentration (MIC) of plant extracts is estimated according to (NCCLS, 2002). The bacteria were gowned at a nutrient agar for 6 hours, then 0.1 ml of airborne bacterial containing 1×10^{16} bacterial cells/ml placed on petri dishes by 200 microns for each dish, stretched well, distributed on sterile discs formed of 5 filter papers of 6 mm diameter and the plant extract placed with different concentrations 1/10 and 1/20 and 1/30 and 1/40 and 1/50 mg/ml, the dishes were incubated at a temperature of 37 °C for 24 hours and the minimum inhibitory concentration were determined as the lowest concentration of the plant extract that inhibits the growth of bacteria.

RESULTS AND DISCUSSION

Chemical analyses of *Calligonum comosum*

Samples of seed and fruit extracts of *Calligonum comosum* using different solvents and

sequential separationas mentioned above are injected in the chromatography analyses device GC.MS to obtain the results. Table (1) illustrates the compounds contained in the seed extract of *Calligonum comosum* through injection time in the device which reached to 22 compounds.

Estimate the effectiveness of antioxidant

The effect of *Calligonum comosum* extracts as antioxidants (Table 2), the results shown that stem extract is the highest effective antioxidant among other parts of the plant, where the percentage of the inhibition of free radicals at the minimum used concentration is 46.85%, it also showed inhibition by 58.94% at concentration of 4mg /ml, and reached its maximum effectiveness in inhibiting free radicals when the concentration was 8 mg/ml, that the percentage of antioxidant reached 71.03%.

It is followed by fruit extract, where the percentage of antioxidant is 42.32% at the concentration of 2 mg/ml and increased graduallyto 54.41% when the concentration was 4 mg/ml, then reached it is maximum value of 65.74% at concentration of 8 mg/ml. The roots extracts was the least effective in the inhibition of free radicals, where the percentage did not exceed the inhibition of 13.85% at concentration 2 mg/ml, and gradually reached to 16.28% atconcentration of 4 mg/ml while it was 17.9% at the highest used concentration of 8 mg/ml . For the plant parts contents of vitamins C and A, it has been shown that the ratio of these two vitamins have a direct proportional to the effectiveness of these parts as antioxidant, where the plant stem had the highest percentage of vitamin A by 1293 mg/100g or approximately twice the fruit content of this vitamin and tripled of roots content. The stem also contained 109.12 mg/100g of vitamin C which is equivalent to three times of the fruits and five times of the roots contents of this vitamin. From these impacts and the results it is now clear that the *Calligonum comosum* and its various parts, especially the stem has a good effectiveness as antioxidant, and this efficiency may be due to that it contains a high percentage of the vitamins mentioned which evidenced by the presenceof direct proportional between these

Table 1. Names of the different compounds presented in the seed extracts of *Calligonum comosum*using chromatographic analyses device GC-MS

No	Compound name	Retention time	Molecular Weight	Chemical Formula
1	Eicosanoic acid	5.51	340	$C_{22}H_{44}O_2$
2	Docosanoic acid	6.51	368	$C_{24}H_{48}O_2$
3	Hexadecanal.2-methyl	6.70	254	$C_{17}H_{34}O$
4	Tetradecanoic acid	8.77	228	$C_{14}H_{28}O_2$
5	n-Hexadecanoic acid	9.65	256	$C_{16}H_{32}O_2$
6	octadecanoic acid	9.99	312	$C_{20}H_{40}O_2$
7	Dodecanal	10.76	184	$C_{12}H_{24}O$
8	Undecanoic acid 10-bromo	12.34	264	$C_{11}H_{21}BRO_2$
9	Methyl tetradecanoate	13.41	242	$C_{15}H_{30}O_2$
10	Hexadecanoic acid 15-methylester	13.93	284	$C_{18}H_{36}O_2$
11	Octadecanoic acid 9.10-dihydroxy methylester.bic (trifiuoroacetate	22.27	522	$C_{23}H_{36}F_6O_6$
12	2-Methyl-z.z-3.13-octadecadienoL	22.43	280	$C_{19}H_{36}O$
13	Pentadec -7-ene.7-bromomethyl	22.94	302	$C_{16}H_{31}BR$
14	9.12.15-Octadecatrienoic acid(acetyioxy)methyl ethyl ester	23.57	436	$C_{25}H_{40}O_6$
15	1-iodo-2-methylundecan	29.60	296	$C_{12}H_{25}I$
16	Hydroxylamine.O-decyl	29.75	173	$C_{10}H_{23}NO$
17	1-Hexacosene	35.4	364	$C_{26}H52$
18	Trifluoroacetic acid .n-octadecyl ester	36	366	$C_{20}H_{37}F_3O_2$
19	10-Bromodecanoic acid.ethylester	7.11	278	$C_{12}H_{23}BRO_2$
20	Undecanoic acid.ethylester	8.87	214	$C_{13}h_{26}O_2$
21	Pentadecanal	11.70	226	$C_{15}H_{30}O$
22	Hexadecanoic acid.ethylester	13.9	284	$C_{18}H_{36}O_2$

Table 2. The percentages of inhibition of stem, fruits and roots extracts of the *Calligonum comosum*as antioxidantand the contents of these parts of vitamins A and C

Biochemical constituents	Calligonum		
	Root	Stem	Fruits
Vitamin A "µg/100g"	319.51±42.9	1243.90±50.8	619.92±33.7
Vitamin A "µg/100g"	21.63±3.5	109.12±6.2	35.12±3.8
Antioxidant Activity (%)			
25µl (contain 50µg of extract)	13.55±2.5	46.85±3.1	42.32±3.5
25µl (contain 100µg of extract)	16.28±2.8	58.94±5.3	54.41±6.2
25µl (contain 200µg of extract)	17.90±1.7	71.03±4.5	65.74±5.7

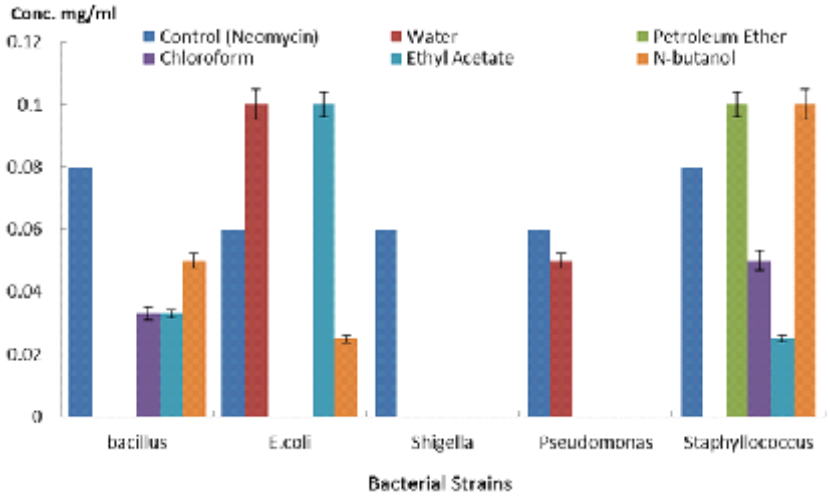

Fig. 1. The minimum inhibitory concentrations (MIC) of the stem extract from *Calligonum comosum* using different solvents on the studied bacterial strains

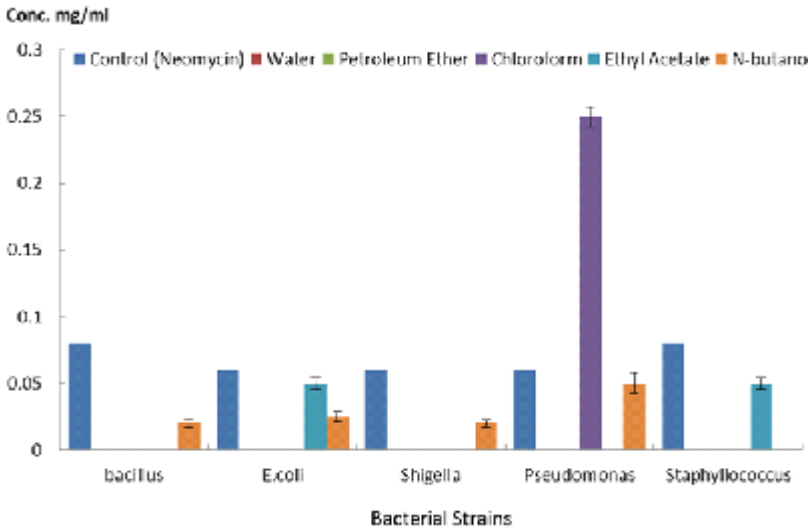

Fig. 2. The minimum inhibitory concentrations (MIC) of the fruits extract from *Calligonum comosum* using different solvents on the studied bacterial strains

vitamins founded in the plant and the percentage of the plant effectiveness on inhabitation of the free radicals. These results are consisted with the results of (Badria *et al*.2007) in her study of the effect of *Calligonum comosum*extracts as antioxidant.

The effect of plant extracts on the bacterial strains

From the compounds that were obtained by chromatographic analysis device GC.MS using several solvents,it showed that a large number of these compounds have acidic nature that effect on the bacteria by ±1gram, where the effect of these extracts on a some bacterial strains were studied by assessing the minimum inhibitory of the stems and fruits extracts from *Calligonum* plant on the studied bacterial strains, for the minimum inhibitory concentration (Figure 1 and 2) shows that the lowest influential concentration inhibits the growth of bacterial strains is founded in the stem of the *Calligonum* plant extract by solvents chloroform, and ethyl acetate on the *Bacillus subtilis* strain, by 0.033 mg/ml. which coincide with the results of (Salma and Marroliki, 2010) in their studies on the effect of minimum inhibitory of the *Polygonum oviculare* plant on a group of bacterial strains of the Family Polginaceae which contains also *Calligonumcomosum* plant. The effect on the strain *Staphylococcus aureus*with a value of 0.05 mg/ml, and the extractobtained by using n-butanol solvent is affecting by the same concentration on the strain *Staphylococcus aureus*, and with solvent Petroleum ether on the strain *Pseudomonas aureus*, followed by the water extractor on the strain *E. coli* by 0.1 mg/ml.

For the minimum inhibitory impact of the neomycin antibiotic, it showed a similar impact on all the studied strains by 0.06 mg/ml, except for *Staphylococcus aureus*, and *Bacillus subtilis*as it reached 0.08 mg/ml, these results illustrate that the stem extract of the *Calligonum* plant by chloroform and Ethyl acetate solvents is the best for bacterial strain of *Bacillus subtilis*, which gave better results than that of the antibiotic used as an indicator where the minimum inhibitory concentration of 0.033 mg/ml, which is equivalent to half of the minimum inhibitory concentration of the indicator of 0.08 mg/ml, and thus for the impact of these two solvents on *Staphylococcus aureus*bacterial strain, where the minimum inhibitory concentration was 0.05 mg/ml, and is considered the best in terms of impact compared to the effect of the antibiotic used as indicator.

The same is for the minimum inhibitory concentration of the stem extract using n-butanol on the bacterial strain *Staphylococcus aureus* while, the minimum inhibitory effect of aqueous and alcoholic extractsof the seed by solvent Ethyl acetate was higher than the minimum inhibitory effect of the indicator that was 0.1 mg/ml.For the fruits extract,the minimum effective concentration by the solvent n-butanol on the strains *Bacillus subtilis,E. coli*, *Shigella* and *Pseudomonas*, this is consistent with the results of (Karuppusamy and Rajasekaran, 2010) in their study of the *Rheum rhapontium* plant extract, which is of the same family of *Calligonum comosum*plant,on a group of bacterial strains include the strains studied in this research except the strain *Shigella*.This is followed by the effect of Ethyl acetate solvent on strains *E. col*, and *Staphylococcus aureus*, and then chloroform solvent effect on the strain *Pseudomonas*.

It is illustrated that the fruit extract by solvent n-butanol is the best in terms of impact on the bacterial strains *Shigella sonnei*, *Bacillus subtilis* and *E. Coli*, where the minimum inhibitory effect was 0.020 mg/ml, which is equivalent to one-third of the minimum inhibitory concentration for the indicator, 0.06 mg/ml. The effect of the stem extract by solvent n-butanol is very wide on these strains, followed in terms of the degree of influence the stem extract by Ethyl acetate solvent on the bacterial strain *Staphylococcus aureus* and *E. coli*, where the minimum inhibitory concentration of these strains was 0.05 mg/ml. The effect of extract by n-butanol solvent on the *Pseudomonas* strain was the lowest recorded impact data with value of 0.15 mg/mlwhich is higher than the minimum inhibitory concentration of the indicator.

CONCLUSIONS

1. To benefit of the effectiveness of *Calligonum comosum* extracts as antibacterial.
2. To benefit of the effectiveness of *Calligonum comosum* extracts as antioxidant in the different fields related to the needsof this effect.
3. To maintain the growth and spread of

Calligonum comosum plant and protect it from the dangers such as timber cutting, overgrazing, and desertification by introducing it to new areas in terms of rebuilding vegetation cover and maintaining it.

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REFERENCES

1. Ackacha, M. A. Removal of Zn(II) Ions from Aqueous Solution by New Adsorbent: *Calligonum comosum*. *Int. J. Chem. Env. Engin.*, 2010; **1**(1): 210-218.
2. Badria, F.A., Ameen, M. , Akl, M.R. Evaluation of cytotoxic compounds from *Calligonum comosum* L. growing in Egypt. Z Naturforsch C, 2007; **62**(9-10): 656-60.
3. Bhuraneswari, K., poongthai, S. G. Kuruvilla, A., Applaraju, B. Inhibitory concentrations of *Lawsonia innermis* Drug powder for urinary pathogenes. *Ind. J. pharmacolo.*, 2002; **34**: 260-263.
4. El-Hawary, Z.M., Kholief, T.S. Biochemical studies on some hypoglycemic agents (II) effect of *Calligonum comosum* extract. *Arch. Pharm. Res.*,1990; **13**: 113-116.
5. Grand, A., Woudergem, P.A., Verportes, R., pousset, J.L., Anti-infection phytotherapies of treesavannah sengl (West-Africa), II-Anti microbial activity of 33 species. *J. Ethnopharmacol.*,1988; **22**:25-31.
6. Karuppusamy, S., Rajasekaran,K.M. A New Approach to High Throughput Antibacterial Screening of Plant Extracts by *Resazurin Redox* with Special Reference to Medicinal Plants of Western Ghats. *Adv. Biol. Res.*, 2010; **4**(4): 201-206.
7. Molyneux, P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J. Sci. Technol*,2004; **26**(2): 211.
8. Nwosu, M.O., Okafor, J.J. Preliminary studies of the antifungal activities of some medicinal plants against Basidiobulus and some pathogenic fungi. *Mycoses*1995; **38**: 191-195.
9. Riadh, H., Imen, F., Abdelmajid,Z., Sinda,F. Detection and extraction of anti-listerial compounds from *Calligonum comosum*, a medicinal plant from arid regions of Tunisia. *Afr. J. Tradit. Complement Altern Med.*,2011; **8**(3):322 327.
10. Salama, H. M.H., Marraiki,N. Antimicrobial activity and phytochemical analyses of *Polygonum aviculare* L. (Polygonaceae), naturally growing in Egypt. *Saudi J. Biolog. Sci.*,2010; **17**: 57-63
11. NCCLS method for broth dilution antifungal susceptibility testing of filamentous fungi. Approved standard M38-A. NCCLS, Wayne, PA. 2002.