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Antibacterial Activity of Different Fractional Extracts of Cerastium glomeratum

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Abstract

Every plant available in the universe is not produced to be the part of biodiversity, but is also produced to obtain medicinal purposes. The medicinal value of the plant is due to the availability of various chemical compounds. Since prehistoric time, the crude extract of plant is used to cure various diseases, although the biologically active components of the plant were unidentified. In the present study the antibacterial activity is performed for various extracts of *cerastium glomeratum*. The plant *cerastium glomeratum* was collected from near kalpani river Mardan, Pakistan and was identified by lecturer Israr department of botany GPGC Mardan, Pakistan. The plant was dried under shade and grinded into powder form. The extraction process was done by using soxhlet apparatus, reflux condenser and maceration process using ethanol as a solvent. The extract was divided into different fractions of n-hexane, DCM, ethyl acetate and water. These four extracts were tested against gram positive (*staphylococcus aureus*) and gram negative (*E. coli*) bacteria using well diffusion technique. The water extract show a wide range inhibition zone against both gram positive and gram negative bacteria while n-hexane, DCM and ethyl acetate fractions show no zone of inhibition against bacteria. In effort to find new antibacterial compounds *cerastium glomeratum* seems to be a good plant for additional phytochemical studies.

Keywords: Antibacterial activity, different fractions of extract, well diffusion, S.aureus, and E.coli

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Introduction

Pakistan is wide country having sea, desert, planes, rivers and mountains thus there is good climate for plants including medicinal herbs. Mardan, a city situated near the river *Kalpani*. Survey of medicinal plants reports reveals that this region is very rich in plant species and several angiospermic species [3-5].

Cerastium glomeratum syn *Cerastium viscosum, Cerastium vulgatum var. glomeratum* is flowering plant belongs to the *Caryophyllaceae* (pink) family commonly known as sticky mouseear chickweed and clammy chickweed. *Caryophyllaceae* family consists of more than 104 genera and above 2000 species [6,7].

Cerastium glomeratum found in some hillstations in Pakistan, which may be came from Eurasia. It is an annual herb 10-35 cm tall. Stem are simple or branched, hairy, often on one side. Leaves are 1-2 cm long, 3-7 mm broad, obovateelliptic to lanceshaped-elliptic, thinly or thickly hairy, with a sharp to dull tip bases. Flower stalk is densely glandular-hairy, shorter or as long as the sepal-cup. Flowers are borne in roundish heads at the end of branches. Sepals are 4-5 mm long, lance-shaped, glandular-hairy, often tinged purple. Petals are white, as long as or slightly shorter than the sepals. The flowering time is April-July [8].

The family *Caryophyllaceae* is the richest source of various phytochemicals but unfortunately a little work is done on *Cerastium glomeratum*. A large number of other compounds viz; fatty acid derivatives, benzenoids, phenyl propanoids, isoprenoids, and nitrogen containing compounds have been reported from the plants belonging to this family [9-13].

Literature survey reviled that only fourteen cyclic glycolipids, named glomerasides A–N, have been isolated from *Cerastium glomeratum* [14] and some fatty acids. [15]

It has been widely used as a folk medicine for various ailments. The juice of *Cerastium glomeratum* was applied to the forehead to reduce headaches, also be drop into the nostrils to cure nose bleeding. Conventionally it is used as diuretic, glucophage, and tonic [16].

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In the present study *Cerastium glomeratum* plant of Caryophyllaceae family is selected for the biochemical investigation.

Experimental

Plant material

The parts of *Cerastium glomeratum* were collected during March 2019 from near the river *Kalpani* of the Mardan District, was identified and authenticated by Lecturer Israr, Botany Department, GPGC, Madan, Pakistan. Fresh or dried plant material can be used as a source for the extraction of secondary plant components. Freshly harvested and dried material is more commonly used, since old, dried material stored for a period may undergo some qualitative changes.

Extraction of plant materials

The powder form of *cerastium glomeratum* was used for extraction process. The extraction process was done by three different methods. i.e. soxhlet extraction, maceration and extraction by reflux condenser. The solvent used for extraction was ethanol. The ethanol extract was then divided into different fractions, these fraction include n-hexane fraction, DCM fraction, ethyl acetate fraction and water fraction. These fractions were dried by using electric water bath and antibacterial activity was performed.

Test organism

For testing antibacterial activity against different fractional extract of *cerastium glomeratum* staphylococcus aureus was used as gram positive bacteria and Escherichia coli as gram negative bacteria. Clindamycin, ampicillin, and kanamycin sulphate antibiotics were used as positive control and dimethyl sulphoxide (DMSO) was used as negative control against these bacteria. Bacterial strain was obtained from Bacha Khan medical college mardan and was cultured in nutrient agar medium.

Antibacterial assay

Antibacterial activity for different fractional extract of *cerastium glomeratum* was investigated

against gram positive and gram negative bacteria by using well diffusion technique. 20mg from each dried extract was dissolved in 1ml of DMSO in order to make 20mg/ml solution. From all of the four fractions 30µl solution was inserted in the well made in agar medium present in Petri plates and incubated at 37°C for 24 hours. After 24 hours the incubated Petri plates was examined and the zone of inhibition was measured with the help of ruler in millimetre. This experiment was repeated three times the result was noted. Mean for zone of inhibition was calculated and verification was done by using one way ANOVA.

Results and Discussion

The measured zone of inhibition along with diameter of the well is shown in table 1, 2 and 3 and figure 1, 2 and 3.

Table 1. Zone of inhibition of Escherichia coli for different extract and clindamycin, ampicillin and kanamycin standard (in mm) Zone of inhibition of different extracts and antibiotics on E.coli

S.No	n-hexane	DCM	Ethyl acetate	water	clindamycin	ampicillin	Kanamycin
1	8	10	8	26	20	15	26.3
2	8	10	8	25.8	20.1	15.4	26
3	8	10	8	26.3	20	15.3	26.1
Mean	8±0	10±0	8±0	26.03±0.1	20.03±0.02	15.23±0.09	26.13±0.07

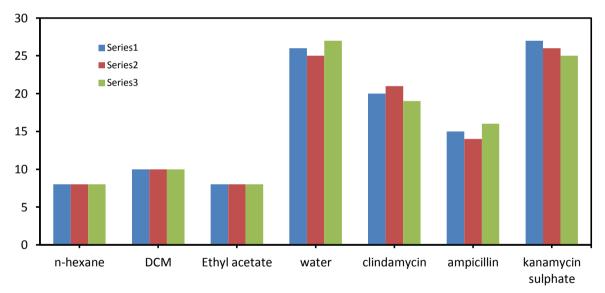


Figure 1 Zone of inhibition of Escherichia coli for clindamycin, ampicillin and kanamycin standard and different extracts (in mm)

Table.2- Zone of inhibition of staphylococcus aureus for clindamycin, ampicillin and kanamycin standard and different extracts (In mm)

	Zone of inhibition of different extracts and antibiotics on S.aureus								
S.No	n-hexane	DCM	Ethyl acetate	water	clindamycin	ampicillin	Kanamycin		
1	8	10	8	28.1	20	15	26.3		
2	8	10	8	28.1	20.1	15.4	26		
3	8	10	8	28	20	15.3	26.1		
Mean	8±0	10±0	8±0	28.06±0.02	20.03±0.02	15.23±0.09	26.13±0.07		

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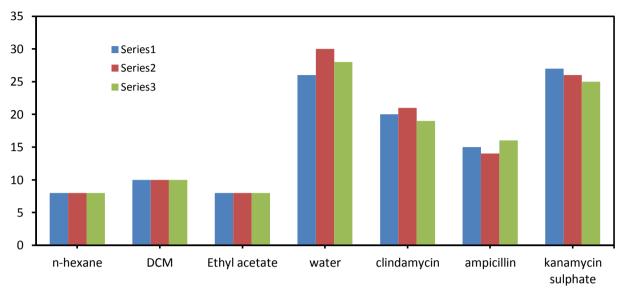


Figure.2- Zone of inhibition of staphylococcus aureus for clindamycin, ampicillin and kanamycin standard and different extracts (in mm)

Mean of zone of inhibition for different extracts and standards									
Organism	hexane	DCM	Ethyl acetate	water	clindamycin	ampicillin	kanamycin		
Escherichia coli	8mm	10mm	8mm	26.03mm	20.03mm	15.23mm	26.13mm		
Staphylococcus aurous	8mm	10mm	8mm	28.06mm	20.03mm	15.23mm	26.13mm		

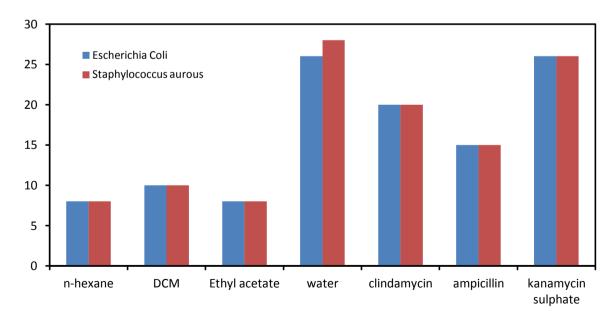


Figure.3- Mean for zone of inhibition of different extract and standards against E.coli and S.aureus (in mm)

The antibacterial activity was performed for various extract of *cerastium glomeratum* and was examined carefully. The inhibitory effect of fractional different extract of cerastium glomeratum is shown in table (1, 2 and 3) and figure (1, 2 and 3) which was tested by using one way ANOVA. The result after measurement of inhibition zone indicates significant antibacterial activity against S.aureus (gram positive bacteria) which is shown in Table.2 and figure.2. While inhibition zone for E.cloi (gram negative bacteria) is shown in table.1 and figure.1. After examining all the four fractional extract of cerastium glomeratum, water extract show a wide zone of inhibition while DCM extract show just a little zone of inhibition and the remaining fractions of extract show no activity against E.Coli and S.aureus given in table.3 and figure.3. The most interesting fact of this activity is that water extract show activity against gram positive and gram negative bacteria, the zone of inhibition for water extract is 26.03 mm for E.coli and 28.06 mm for S.aureus. In both bacteria, the water extract have 2 mm greater zone of inhibition for S.aureus than E.coli presented in table.3 and figure.3. The antibiotics used as positive were Ampicillin, clindamycin control and kanamycin sulphate during the experiment. These antibiotics give zone of inhibition from 15-27 mm. After comparing the zone of inhibition for antibiotics and result obtained from water extract show greater activity than the antibiotics used as positive control shown in table.3 and figure.3, which is another interesting feature of water fractional extract from *cerastium glomeratum*. This water extract contain many chemical compounds which is responsible for showing activity against both gram positive and gram negative bacteria. If these chemical compounds are separated and tested further we will find individual antibacterial compound for both gram positive and gram negative bacteria which will be better than other antibiotics.

Conclusion

After performing antibacterial activity for different extracts of *cerastium glomeratum* we concluded that, in order to find out antibacterial compounds the best choice is the water extracts of *cerastium glomeratum*. The water extract of *cerastium glomeratum* has three possibilities for antibacterial compounds. The first possibility is that it may contains compounds in which one compound show activity against gram positive bacteria and another compound show activity against gram negative bacteria. The second possibility is that the water extract of *cerastium glomeratum* may contain compounds in which only one compound show activity against both gram positive and gram negative bacteria. The third possibility is that there may be more than one compounds which show individual activity for both gram positive and gram negative bacteria.

Keeping this conclusion in mind we are interested for further working on *cerastium glomeratum*.

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