IN VITRO ANTIOXIDANT AND ANTIBACTERIAL POTENTIAL OF MEDICINAL PLANT CERASTIUM FONTANUM

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***Abstract*— Naturally God gifted medicinal plants which contain more potentially active compounds their characterization and isolation are very important and can provide us a great help in making new drugs to cure many diseases. Our current attempt was made to obtain the ethanolic extracts of medicinal plant Cerastium fontanum using a hot continuous soxhlet process and also via maceration method. The concentrated dried fraction extracts of Cerastium fontanum such as aqueous, n-hexane, ethyl acetate and dichloromethane were achieved using liquid-liquid extraction which were then evaluated for in vitro antioxidants potential using DPPH (1,1-diphenyl-2-picryl-hydrazyl) assay and also agar-well diffusion method were used for antimicrobial potential. In vitro antioxidants inhibition potential were measured through spectrophotometer at various concentration (500 to 62.5µg/mL) prepared in standard solvent. Among all extracts aqueous fraction showed IC50 = 2.9 ± 0.05mg/mL maximum potency towards stable DPPH much closer to standard control acarbose IC50= 2.61± 0.01mg/mL. The antibacterial activity results indicated that all fractions found active against both strains (gram positive and gram negative) of bacteria. In various extracts only the aqueous fraction extracts showed excellent inhibition potential against Staphylococcus aureus and Escherichia Coli. While dichloromethane fraction extracts were found active only with gram negative strain. Thus, the Cerastium fontanum extracts possess much higher inhibition potential then standard available antibiotics in the market. So, it is important to purify and isolate the bioactive compounds present in this plant to design new antibiotics drugs.**

***Keywords*— Extraction, Maceration, Microbes, Antioxidants, DPPH.**

I. INTRODUCTION

To the universe naturally God gifted medicinal plants which contain more potentially active compounds, and their characterization and isolation are essential and can provide us a great help in making new drugs to cure many diseases (Hassan and Ullah, 2019). The human utilized plant species according to their needs such as for foods, medicinal purposes and chemical practices and other health related products. For medicinal and food requirement, the plants were tested and categorized into poisonous and nonpoisonous species (Zaidi, 1993, Al-Snafi, 2016). Plant based drugs are easily obtained and rarely have no side effects and are safe effective and also inexpensive (Javid *et al.*, 2015). In the early days, phytochemicals were used in the form of herbal and homeopathic medicine for the treatment of various diseases because these substances show protective and preventive properties against diseases (Smadi, 2011). Throughout the world, people utilized plants, vegetables products and herbs as folk medicines. Women for family care used medicinal plants at home. According to International Trade center (ITC), the imports and starting materials of plant origin in cosmetics and pharmaceutical industries have the value of the order of 52.9 million USD. In 1971, the value grew to 71.2 million USD and then steadily grew annually at the rate of 5-7% to the mid 1980s (Holley and Cherla, 1998). The common medicine used around the world in our daily life contains important compounds about more than 25% extracted from plants. In the United States (US), 10% of all directed drugs are primarily extracted from medicinal plants, because they contain primitive components and are very effective (Borde *et al.*, 2014). For therapeutic treatment, usage of raw extracts from plants with known antimicrobial action study conducted in various countries in the last few years due to achieving good result in this area (Bekele and Hazare, 2017). More than 200000 compounds are isolated from natural products throughout the world. From the last few years, the research on plant-derived drugs and natural products is significantly rising and Fabricant and Farnsworth isolated 122 compounds from 96 plants, which are widely used in medicine (Puspita, 2015). According to (Naczk and Shahidi, 2006) research studies, about 20% of plants are used in a positive way to treat harmful diseases. USA has directed more than 100 drugs, which are isolated from natural sources while 90% of those isolated from plants and purified. In recent days, more than 50% anticancer medicines are isolated from plants. At the end of 20th century, about a hundred different anticancer drugs were developed in which 25% were derived from natural products. In United States, plant extracts constitute 25% of total drugs, while in fast developing countries such as China, the contribution of plant-derived drugs is more than 75% (Smadi, 2011). According to Rana and Samant (2011), Cerastium fontanum plant species is used for lowering the rise of temperature in body, that is fever, for the treatment of upper respiratory infections and used in cough. While Khan (Sher *et al.*, 2011) worked on the ethno botanical studies of Cerastium fontanum using a whole plant and reported that this species could be used

as a refrigerant. From medicinal plants, extraction or isolation produces some potent compounds or substances that are accountable for a biological activity (Kibwage *et al*., 2005). The modern medicine, food-supplement, folk, and traditional medicine are mainly obtained from plants (Hammer *et al.*, 1999). In medicinal plants, important constituents such as vitamins A, C, E, flavonoids, lignin's, and tannins, phenolic compounds are found and represent antioxidants potential (Suffredini *et al.*, 2004). Our current study were evaluated on the medicinal plant Cerastium fontanum for extraction, fractionation, and performing their in vitro antioxidants 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and antimicrobial (against gram-positive and gram-negative strain) activity.

II. METHODS

A. Identification of Plants

The plant species Cerastium fontanum were identified and confirmed by a botanical expert, Prof. Muhammad Israr of Botany department, Government Post Graduate College, Mardan, 23200 KP, Pakistan, and through various flora and literature survey comparison and studies.

B. Collection of Plant Material

The plant species Cerastium fontanum was collected as a whole plant during the flowering stage from a fertile land at village Muhib Banda and its graphical coordinates are 34° 9' 39" North, and 72° 19' 23" East outside district Mardan 23200 Khyber Pakhtunkhwa, Pakistan during March-April 2019. After collection of the plant washed with sterilized water to eliminate the impurities and soil stuck from its roots and other parts.

C. Drying and Grinding of Plants

After cleaning, the collected plant was cut into small pieces and then stored it for drying in the shade to protect it from direct sunlight and other impurities and contamination from outside environment to avoid the plant constituent's decomposition and other harmful effects. The plant was well dried for about 2-3 weeks and then converted into uniform size powder form by using a grinder for better extraction process.

D. Plant Extraction-Soxhlet Extraction

For extraction using soxhlet apparatus, 30 gram of finely powdered plant was kept in a porous bag made from cellulose were manually prepared and then inserted into the thimble of the soxhlet chamber for the extraction process. In the bottom flask of soxhlet, 250 mL ethanol were kept for extraction which was heated and evaporated by a mantox heater at moderate temperature around 400C. The evaporated ethanol was condensed by recycling making the upper chamber of soxhlet as a condenser by introducing water inflow and outflow through the outside chamber of soxhlet and condensation of solvent was continuous falling on the plant sample and extraction occur with it. This process was done for 36 hours and ethanol extract was obtained. The ethanol extract was then kept in a controllable water bath to evaporate ethanol from it and solid plant extract was achieved which were fractionated again to dichloromethane, water, n-hexane and ethyl acetate and concentrated out for different biological activities.

E. Maceration

In this extraction method, 30 grams of powdered plant sample was taken in a Pyrex glass jar and 300 mL ethanol were added to it. It was allowed for about 24 days with proper shaking done on a daily basis. The extract was obtained through soaking and filtering it by using paper filter paper. The ethanol extract was then kept in a controllable water bath to evaporate ethanol from it and a solid plant extract was obtained. Then the dissolved solid plant extract in distilled water, which were treated with different solvents like ethyl acetate, dichloromethane, and n-Hexane and the extract was separated in different fractions and concentrated out for different biological activities.

F. Antioxidants Activity of DPPH

All extracted fractions were obtained in a good yield and were employed for antioxidant potential under 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay according to the previous method reported by Hossain *et al.* (2011), Jacinto *et al*. (2011), Marinova and Batchvarov (2011), Hassan *et al*. (2019). In the first stage, all entire dried fractions water, dichloromethane, n-hexane, and ethyl acetate were dissolved in DMSO (Dimethylsulfoxide) solvent in a serial by half dilution to (62.5, 125, 250, and 500)µg/mL. The standard solution of DPPH was prepared by dissolving (0.0238) gram DPPH by weight in 100 mL solvent and protected from light decomposition with aluminum cover and were stored under room temperature in dark 2 hours prior to the assay setup. In the second steps, each extracted fractions were treated separately with DPPH solution (90 µL) and sampled 10 µL with concentrations (62.5, 125, 250, and 500) µg/mL. Then employed for incubation period 25-30 minutes at temperature (370C). Absorbance values for each sample taken at a fixed wavelength (517 nm) in triplicate. The AA (ascorbic acid) were used as positive controls and were prepared in the same concentration as above and explored with DPPH. While in the negative control, Dimethylsulfoxide solvent media were placed. Control Abc (is the absorbance of standard) and Sample Abc (is the absorbance of the compound).

The equation of Rehman *et al.* (2018), Khan *et al*. (2017) was used for the calculation of DPPH free radical scavenging activity and was measured in percent inhibition.

$$\text{DPPH radical scavenging activity (\%)} = (\text{Control Abc} - \text{Sample Abc})/(\text{Control Abc}) \times 100$$

G. Antibacterial activity-Preparation of Solution from Fractions Extracts

The dried four fractions were dissolved in DMSO (Dimethoxylsulfoxide) at a concentration of 10mg/mL to make a solution. The solution was kept in a centrifuge for 30 minutes at 12000 rpm for better mixing. Standard antibiotics Gentamicin (10mg/disc), Ampicillin (10mg/discs), and Ofloxacin (1mg/ml) were used for comparing the activity of each fraction to standard antibiotics.

Table 1. DPPH activity results of different fractions of *Cerastium fontanum* plant.

S. No	Samples Extracts	IC_{50} mg/mL
1	Water Fraction	2.9 ± 0.05
2	Ethyl Acetate Fraction	3.5 ± 0.02
3	*n*-Hexane Fraction	6.37 ± 0.2
4	DCM Fraction	4.94 ± 0.05
5	Control Ascorbic acid	2.61 ± 0.01
6	Control Propyl Gallate	1.6 ± 0.05
7	Control TBH (3-Tert-butyl-4-hydroxyanisol)	1.2 ± 0.1

The data represented as Mean via statistics as a Standard deviation (SD) in triplicate under standard graph pad prism software Mean ± SD. The Median inhibitory concentration plotted concentration with percent inhibition.

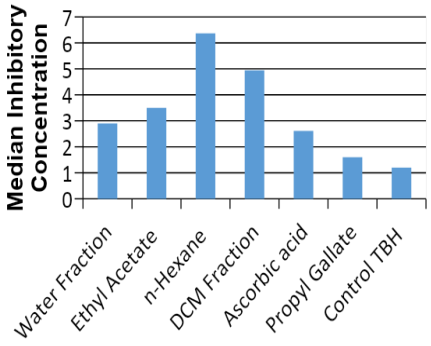

Figure 1- Comparison of antioxidant potential of extracts using stable DPPH.

H. Microbes Used in Test

Two types of bacteria, Escherichia Coli (gram negative) and Staphylococcus aureus (gram positive), were used for antibacterial activity. Both bacterial strains and antibiotics used during the experiment were obtained from Bacha Khan Medical College, Mardan, 23200 KP, Pakistan.

I. Culture Media Preparation

For antibacterial activity, MHA (Muller-Hinton Agar) was used for bacterial media preparation. Culture media was prepared by dissolving 9.5g MHA in 250 ml distilled water. The obtained solution was mixed properly and for better dissolution it was boiled with frequent agitation and opalescent gel was obtained. Then the media was autoclaved for sterilization at 15 lbs pressure and at a temperature of 121 degrees C. Then the sterilized media was allowed to cool down in a laminar flow hood at room temperature. Then pour 25 mL of media in each Petri dish and leave it for a while until it becomes solid. After solidification, spread cultural bacterial microbes on each dish. Make 6 wells in each Petri dish at a distance of 2.5 cm from each other. The 30µL of each fraction was poured in the first four bores, antibiotic in the second last, solvent was poured in the last well, and antibiotics discs were also. Store Petri dishes for incubation in a biological oxygen demand (BOD) incubator at 37°C. After 24 hours, the samples were taken from the incubator and the zone of inhibition were measured in millimeters for each extract and standard antibiotic.

III. RESULTS

A. Antioxidant Activity

Natural antioxidants are currently demanded due to less or no side effects and are very effective and efficient as compared to synthetic antioxidants, and were employed in pharmaceutical, health and cosmetics related products. The *in vitro* free radicals and scavenging ability is shown in Table 1 and Fig. 1. In all extracted fractions of *Cerastium fontanum*, the maximum potential of DPPH were shown by the water fraction having IC_{50} = 2.9±0.05 which is very much closer to standard acarbose having IC_{50} = 2.61±0.01 percent as shown in Table 1 and Fig. 1. All other fractions were also found active during the activity, secondary ethyl acetate fractions possessing IC_{50} = 3.5±0.02 while dichloromethane showed IC_{50} = 4.94±0.05 percent inhibition potential as shown in Table 1. While other controls found more active towards a stable DPPH such as Propyl Gallate having IC_{50} = 1.6±0.05 and TBH $IC5_{0}$ = 1.2±0.1 percent as shown in Table 1 and Fig. 1. The purification of these extracted fractions may enhance their potential towards a stable DPPH because they contain flavonoids which are accountable for antioxidants potential.

In antioxidant activity, two reactive species are known, one is reactive oxygen species (ROS) and the other is reactive nitrogen species (RNS), both are recognized to damage the enzymes, nucleic acid, protein, and lipids which may be the main cause of the cell and tissue injury. However, oxidative stress and mainly free radicals are the major cause of a vast range of degenerative and death causing diseases including cancer, Alzheimers, diabetes, liver injury, atherosclerosis, and coronary heart disease. From several evidences, we can determine that the main cause of cell and tissue injury is oxidative stress (Veerapur *et al*., 2009, Lü *et al.*, 2010, Sánchez *et al*., 2010). The term oxidative stress means that the cell and tissues have an oxidant status which may be changed by exposure to oxidants. During oxidative stress, the depletion or loss of antioxidants occur. The ROS and RNS contain diverse reactive entities such as superoxide (O_2), hydroxyl (OH*), peroxyl (ROO-), peroxynitrate (ONOO) and nitric oxide (NO) radicals, while the non free radicals species like hydrogen peroxide (H_2O_2), nitrous oxide (HNO_2), and hydrochlorous acid (HOCl) (Mavi *et al*., 2004, Mosquera *et al*., 2007, Manandhar, 2002). Interestingly, a helpful mechanism is developed by aerobic organisms, which is an antioxidant defense mechanism that captures the damage caused by ROS and RNS entities. The defense mechanism can be both enzymatic as well as non-enzymatic.

In the enzymatic defense mechanism, various types of enzymes such as reductase, dismutase, peroxidase, sueroxides, and nitric oxide synthase are included. While non-enzymatic mechanisms are deterred or restricted to antioxidants and trapping, agents such as flavonoids, uric acid, vitamin k, glutathione, cysteine, albumin, and bilirubin. It also included trace amounts of elements like zinc and selenium (Braca *et al*., 2002; Maxwell, 1995; Schi-

nor *et al*., 2007; Mosquera *et al*., 2007). Along with natural antioxidants, various addition or synthetic antioxidants have also been reported such as butylated hydroxyanisole and butylated hydroxytoluene. It has been reported that synthetic antioxidants have high risk of side effects (Koduru *et al*., 2006); therefore, give proper attention towards natural antioxidants. Interrogations on identifying natural antioxidants has become an important problem (Alma *et al*., 2003). In the last few years, natural antioxidants reproduce and considered as a good preventive medicine (Govindarajan *et al.*, 2003). Plants are a rich source of antioxidants and every year thousands of new antioxidant compounds have been invented. Plants are considered as a potential source of new compounds having antioxidants properties (Mosquera *et al.*, 2007; De Lencastre *et al*., 2007; Mishra *et al*., 2007). A stable free radical such as the compound of diphenyl picrylhydrazyl is demonstrated by the goodness of the delocalization of the free electrons over the whole molecule. A deep violet color as a result of delocalization followed by absorption band in the solution of methanol at about 517 nm. The substance which can donate a hydrogen atom, is mixed with the DPPH solution, then it gives the deduct form with the vanish Violet color. At the end pale color is indicated due to the presence of pecryl residue (Molyneux, 2004; Kanatt *et al*., 2018; Kanwal *et al*., 2018; Bahramian *et al.*, 2018). It is concluded that their potential towards a stable DPPH because they contain flavonoids which are accountable for antioxidants potential.

B. Antibacterial Activity

The current was made due to resistant development of bacteria to available drugs present in the market. Antibacterial screening was done through agar diffusion method according to Wayne (2011). Extracted antibiotics are very safe and are out of danger such that they have no side effects (Rehman *et al.*, 2015). Uses of active phytochemicals are responsible for biological activity because it can help in the discovery of new drugs (Sharifi-Rad, 2016; Alghazeer *et al*., 2012; Edziri *et al.*, 2010). This current investigation was done for the antibacterial potential of the medicinal plant *Cerastium fontanum* for the first time.

The antibacterial report of *Cerastium fontanum* fractions is summarized below in Table 2.The crude water extract is more potent and showed a greater zone of inhibition for both bacterial strains *Staphylococcus aureus* and *Escherichia coli*. The water extract of *Cerastium fontanum* showed 31.1 ± 0.2mm maximum zone of inhibition for *Staphylococcus aureus* and for *Escherichia coli* it showed the maximum zone of inhibition of 30 ± 0.5mm at 30μL concentration as shown in Table 2 and Fig. 2. The DCM crude extract showed a maximum zone of inhibition of 27 ± 0.5mm for *Escherichia coli* and 14.3 ± 0.2mm of maximum zone of inhibition for *Staphylococcus aureus* at 30μL concentration as shown in Table 2 and Fig. 2.

The ethyl acetate fraction showed a maximum zone of inhibition of 6.6 ± 1mm for *staphylococcus aureus* and for *Escherichia coli* it showed the maximum zone of inhibition of 6.3 ± 0.2mm at 30μL concentration as shown in Table 2 and Fig. 2. The n-hexane fraction showed very less zone of inhibition of 1.5 ± 0.5mm for *staphylococcus aureus* and 1.8 ± 0.2mm maximum zone of inhibition for *Escherichia coli* at 30μL concentration as we can see in Table 2 and Fig. 2. The most virtual and implicit activity of crude extracts obtained from plants against both gram-negative and gram-positive bacterial strains depend on the cell wall of microbes. In gram-negative strains, the efflux system is more responsible for activity (Valsaraj *et al*., 1997; Zampini *et al*., 2009; Li and Nikaido, 2009).

Due to the difference in composition and structure of both bacterial strains, they have different sensitivity (Lambert, 2002). Gram-positive strain is reported that it has high potential activity for different plant species than gram-negative bacteria strain (Afolayan, 2003; Nair and Chanda, 2007a;b; Yaghoubi *et al.*, 2007; Turker *et al*., 2009; Karakas *et al*., 2012). The potential sensitivity of *Staphylococcus aureus* is due to the structure of its cell wall and cell membrane (Zaika, 1988). Gram-negative

Table 2. Antibacterial Potential results of *Cerastium fontanum* fraction extracts.

Fraction Extract	Q	*Staphylococcus aureus*	*Escherichia* coli
Water Fraction	30μL	31.1 ± 0.2mm	30 ± 0.5mm
Ethyl Acetate Fraction	30μL	6.6 ± 1mm	6.3 ± 0.2mm
n-Hexane Fraction	30μL	1.5 ± 0.5mm	1.8 ± 0.2mm
DCM Fraction	30μL	14.3 ± 0.2mm	27 ± 0.5mm
Ofloxacin *Positive control*	1mg	20 ± 0.5mm	11.5 ± 0.15mm
Ampicillin *Positive control*	10mg	15.5 ± 0.3mm	------
Gentamicin *Positive control*	10mg	------	11.9 ± 0.4mm
DMSO *Negative control*	30μL	1 ± 0mm	1 ± 0mm

Q=quantity

The data represented as Mean via statistics as a Standard deviation (SD) in triplicates Mean ± SD. The inhibitory zone calculated in mm for each fraction.

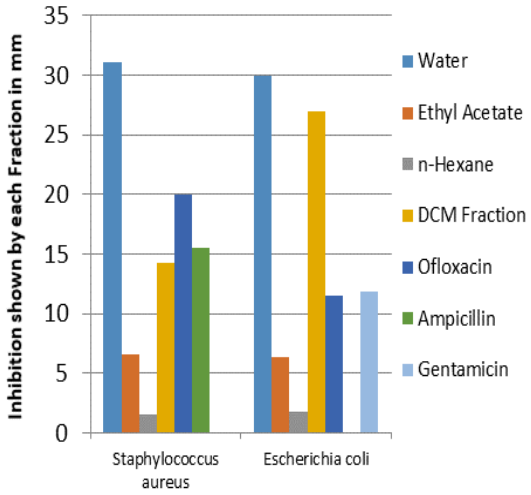

Figure 2-Antibacterial potential of Cerastium Fontanum. Activity of fraction extracts against various strains.

bacterial strains have an impermeable membranes outside which show resistance to antibiotics (Bockstael and Aerschot, 2009). Plants show antimicrobial activity because terpenoids, saponins, steroids, flavonoids, and some other bioactive compounds are present in plants (Mamtha *et al.*, 2004). The presence of flavonoids increases the potential of antibiotics against microbes (Sato *et al*., 2004; Cushnie and Lamb, 2005). The cell wall and cell membrane are weakened by terpenoids (Hernández *et al*., 2000). Saponins leak enzyme proteins from cells of microbes (Zablotowicz *et al*., 1996). From the current investigation it is concluded that this plant may possess an important phytochemical constituent which are responsible for their highest antibacterial activity as compared to standard antibiotics, their activity rate may be decrease or enhanced with purification and isolation of the active compounds from this plant.

IV. CONCLUSIONS

From the current investigation we have concluded that among all extracts aqueous fraction showed IC_{50} = 2.9 ± 0.05mg/mL, maximum potency towards stable DPPH much closer to standard control acarbose IC_{50} = 2.61 ± 0.01mg/mL. The antibacterial activity results indicated that all fractions found active against both strains (gram positive and gram negative) of bacteria. In various extracts, only the aqueous fraction extracts showed excellent inhibition potential against *Staphylococcus aureus* and *Escherichia Coli*. While dichloromethane fraction extracts were found active only against gram-negative strain. Thus, the *Cerastium fontanum* extracts possess much higher inhibition potential then standard available antibiotics in the market. Therefore, it is important to purify and isolate the bioactive compounds present in this plant to design new antibiotics drugs.

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