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Antibacterial potential test for *Scheonoplectus Triqueter (L) Palla*

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ABSTRACT

Our present investigation aims to test the antibacterial action of *Scheonoplectus triqueter (L) palla* against the gram negative bacteria i.e. *Escherichia coli* and gram positive bacteria i.e *staphylococcus aureus*. The crude extract is taken in ethanol which is then fractionated into a different fraction in n-hexane, ethyl acetate, di-chloro methane (DCM) and water.The fractions are then separately applied to *E.coli* and *s.aureus* bacterial strains. Ampicillin (10 micro gram), Clindamycin (2 microgram) and Kanamycin sulfate (30 microgram) are used as a positive control while di-methyl sulfoxide (DMSO) is used as negative control. The result of antibacterial activity of *Scheonoplectus triqueter (L) palla* revealed to us that n-hexane section of the crude extractdisplayed relatively highest activity for *E.coli* (gram negative bacteria) and ethyl acetate fraction displayed relatively high activity for *S.aureus* (gram positive bacteria).

Keywords: *Scheonoplectus triqueter (L) palla; crude extract; fractionation; antibacterial activity.*

1. INTRODUCTION

There are about 258,650 types of species of the upper plants in which only 10% are used as medicine to treat different infections or diseases of ailing communities. The following major problems encounter to generation of income, preservation and farming to the plants that are used as a medicines in Pakistan are the Potential of curative plants of Pakistan, Increased worldwide demands of herbal medicines, reasons of some problems to the existing curative plants of Pakistan, preservation of medicinal plants and case study and policy problems in order to promote the cultivation in huge scale[1]. Human used different therapeutic plants in order to cure many diseases over the centuries. Medicinal Plants are very important throughout the world which is use to treat a number of diseases using plant extracts at first stage of infection [2]. The taxonomist didn't give the desired attention to the family Cyperaceae although this family containsa significant number of monocotyledon plants [3]. Cyperaceae are the halophytic plants found in Pakistan most species of the Cyperaceae are fibrous [4]. Scheonoplectus is a genera of the Cyperaceae plants family having about 60 species distributed all over the world in which 11 species are found in Pakistan [5].

About forty-nine compounds are isolated from the Scheonoplectuslacustris in alcohol and water and all of the compounds are separately applied to the Green Algae i.e. *Selenastrum capricornutum* (a unicellular organism that is normally usedin the tests of toxicity as a bioindicator of eutrophic spots) in order to test the phytotoxic effect of all the forty-nine compounds isolated from *Scheonoplectus lacustris*, of all these compounds the utmost active compound was*(−)catechin* displaying similar inhibition to the Algaecide copper sulfate [6].

The *Scheonoplectus triqueter (L) palla* do not contain any anti-fungal compound because it inhibits the following fungus (a) *Pestalotia Palmarum Cooke* (b) *hyalotialateripes* (Ellis & Ev) Guba, (c) *Polyschema olivacea* (Ellis & Everh), it acts as a host for these fungies [7]. The taxonomy of the S.triqueter may be as follow, kingdom plantae, phylum is Tracheophyta, the class is Liliopsida, order is Cyperales, family is Cyperaceae, genera areScheonoplectus and specie is *Scheonoplectus triqueter (L) palla*.The Scirpustriqueter plays a very economical role to degrade the pollutants that areoriginated from diesel in the wetland.

The experimental data revealed to us that HYRE wetland isolated hydrocarbon degrading microorganism (HDMs) has a significant degrading effect on soil pollutants. A community of HDMs and STL indicated an incredible capacity to degrade HCs component of diesel. For example.15000 mg / kg concentration of diesel, the removal ratio was 72.62% and 67.41% in the STL MS and HDMs respectively [8].There was no report on the antibacterial activity of *Scheonoplectus tiqueter* while some literature is available on the antifungal activity of *S.triqueter* so my main objective in this research paper is to study the anti-bacterial activity of the *Scheonoplectus triqueter* (L) palla. M.F Fay *et al.,* 2003 reported that the samples of Scheonoplectus studied from river of medwey, Tamar with the help of AFLP genetic fingerprint to examine the basic of species of *S.triqueter* to *S.lacustris* and *S.tabernaemontani.* The dominant specie found in *Scheonoplectus* was *S.lacustris* during genetic fingerprinting.
(especially between *S.triqueter* and *S.tabernaemontani*) in all type of situations. While the specie *S.tabernaemontani* is the hybrid in this family which possess too much phenotypic plasticity. [9].

Pedro Jimenez Mejias *et al*., 2007 reported that the *Schoenoplectus corymboses* (Cyperaceae) is vastly distributed mainly in Pakistan, Africa and India. This species also reported in wetland of Morocco, Spain and also found at western extreme of the Mediterrian.
It mainly grows in visited or picnic areas such as Deanna National Park [10]. Eric H.Hoalson *et al.,* 2008 reported that the family Cyperaceae shows a waste variation in chromosome numbers both within the genera. Recently update have reported 4231 chromosome numbers tally in the family. Approximately only

16% species have been currently recognized. We observed avariation within the genera where a maximum possible numbers of tally had been made. Count distribution within the genera is the proof for both agmatoploid and polyploid chromosome numbers variation[11].

Xiaoyan Liu *et al*., 2010 in this research paper they have studied the Scirpus triqueter (*Scheonoplectus triqueter (l) palla*) which is a specie from the family (Cyperaceae) play a very economical role in the degradation of diesel originated pollutants in the wetland. The process of degradation was conducted to restore the pollutants in soil. The experimental data showed that hydrocarbon degrading microorganisms (HDM's) isolated from HYRE wetland has a degradation effect on soil pollutants. A community of STL and HDM's indicated or showed an incredible capacity to degrade HC's component in diesel. For example15000 mg / kg diesel concentration the removal ratio was 67.41%and 72.62 in the HDM's and STL MS respectively [8].

Riffat Batool *et al*., 2013 reported that three species of *Sheonoplectus* were obtained from three different areas of Punjab.

All of the species were introduced to salt stress. *Scheonoplectus triqueter* have advanced root anatomical adaptation for its best/better growth under very crucial high saline condition. When there is an increase in epidermis thickness, cortical cell area, Meta xylem area, phloem area etc in roots were difficult for water loss checking and increased water storing capacity. *S.triqueter* has a very high aerechyma area for gas exchanging and a bulk salt movement. An increase in the number and thickness of cortex and area of vascular tissues lead to a difficulty for its better growth under very hash saline environment [12]. Our present investigation aims totest the antibacterial action of *Scheonoplectus triqueter (L) palla* against the gram negative bacteria i.e. *Escherichia coli* and gram positive bacteria i.e. *staphylococcus aureus*.

2. MATERIALS AND METHODS

The present investigation is conducted on the species *Scheonoplectus triqueter (L) palla* of the genera Scheonoplectus. The plant was collected in the winter season from the Mardan district KPK Pakistan. Only the powdered stem of the *S.triqueter* is used for antibacterial activity.Three types of antibiotics are used as a standard i.e. Ampicillin (10 microgram), Clindamycin (2 microgram) and Kanamycin sulfate (30 microgram).

The solvents are used for extraction of crude from plant, fractionating purposes and dissolution of different fractions in DMSO to apply it tobacteria. The following solvents used are Ethanol (C_2H_5OH), n-hexane (C_6H_{12}), water (H_2O), ethyl acetate (C_2H_5COOH), DCM (di-chloro methane) and DMSO (di-methyl sulphoxide).The plant extract wasapplied on two types of bacterial strains i.e gram negative bacteria (*EscherichiaColi*) and gram positive bacteria (*Staphylococcus aureus).*

2.1. Extraction.

The identification of *Scheonoplectus triqueter (L) palla* was done by the taxonomist professor Muhammad Israr of GPGC Mardan collected in the winter season. Theplant is dried in the shade for 30 days. The dried stem (100 grams) is then grinded in the grinder. The crude extract of60 grams powdered plantis taken in 900 ml of ethanol in 1 liter soxhlet apparatus. The extract is then evaporated from which 8.958 grams of solid crude extract is obtained. The crude extract was then dissolved in 100 ml of water which is then fractionate between water and n-hexane, then the n-hexane fraction is again fractionate between n-hexane and ethyl acetate and at last the n-hexane fraction is fractionate between n-hexane and DCM. In the entire above fraction the water fraction contains the highest quantity of the crude extract i.e 3.65 grams, ethyl acetate contains 1.62 grams, DCM contains 1.178 grams and n-hexane contains 1.928 grams.

2.2. Antibacterial Assay.

The media is prepared in Moller Hinton agar (MHA) for growing the bacterial strain, 9.5 grams of MH agar was taken in 250 ml of distilled water [13-23]. The media and the empty Petri dishes wereautoclaved at 121^0c for about one hour to sterilize the media and Petri dishes. The media was then cooled at room temperature and then it is poured into twelve Petri dishes i.e 20 ml to each Petri dish. The media was then let to solidify and then two types of bacterial strains were applied to the Petri dishes, six plates were strained with *E.coli* bacteria and six plates were strained with *staphylococcus aureus* bacteria. The bacteria is lawned with the help of sterilized cotton swabsand then well are formed in ten plates i.e five of *E.coli* bacterial strain and five of staphylococcus bacterial strain by means of sterilized micro syringe, the well size was 7 mm, the remaining two plates were left for standard antibiotics which weretested by disk diffusion method. Then 20 micro liter of each of four different extracts and DMSO (negative control) were applied to ten plates while three types of standard antibiotics (positive control) were applied to the remaining two Petri dishes i.e Ampicillin (10 microgram), Clindamycin (2 microgram) and Kanamycin sulfate (30 microgram).The Petri dishes werethen packed with parafilm and then stored in the incubator for minimum time of 24 hours, the growth appeared after 24 hoursthen the inhibition zones of treatments (extracts), positive control (antibiotics) and negative control (solvent) were measured.

3. RESULTS

3.1.Extraction and Fractionation.

Scheonoplectus triqueter (L) palla is a halophytic plant which is not too much concentrated with phytochemicals. About 60 grams of the powdered plant stem is taken for extraction in 900 ml of ethanol from which we obtained 8.958 grams of the solid crude extract which is then fractionated so the water fraction contains the highest quantity of the crude extract i.e. 3.65 grams, secondly, the n-hexane containing a high quantity i.e. 1.928 grams, thirdly most concentrated is ethyl acetate contain 1.62 grams and at last DCM containing 1.178 grams of crude extract. All these results are presented in the Figure 1.

3.2. Antibacterial Activity.

The antibacterial activity of different extracts, negative control (DMSO) and standards show that the inhibition zones of the standard antibiotics are more significant relative to the

treatments and negative control. The mean of zone of inhibition is shown in the Table 1 and Figure 2.

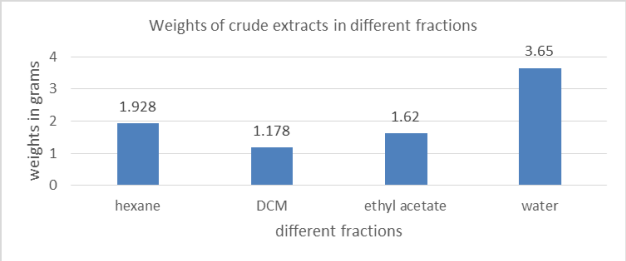

Figure 1. Weights of Crude Extracts in different Fractions.

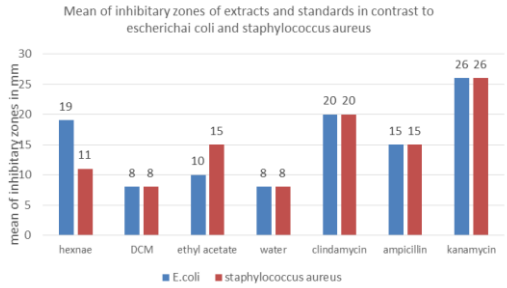

Figure 2. Mean of the inhibitory zones of different extract and standards in contrast to *E.coli* and *S.aureus.*

E.coli (gram negative): For *E.coli* the kanamycin antibiotic showed the highest zone of inhibition i.e 26 mm while the n-hexane fraction (treatment) showed relatively high zone of inhibition i.e 1 mm which is higher than ampicillin antibiotic i.e 15 mm, secondly the relatively high zone of inhibition showed by ethyl acetate i.e 10 mm while the water and DCM both fractions showed 8 mm inhibitory zone.

Staphylococcus aureus (gram positive): Kanamycin antibiotic is the most significant antibiotic for s.aureus showing 26 mm inhibition zone. For *staphylococcus aureus* the fraction ethyl acetate show relatively high zone of inhibition i.e 15 mm which is similar to ampicillin antibiotic which also showing 15 mm inhibition zone, for *S.aureus* the n-hexane fraction showed 11 mm inhibitory zone while the DCM and water fractions showed 8 mm of inhibition zone.

The ethanolic extract of the stem of *Scheonoplectus triqueter (L) palla* is taken through soxhlet extraction method. About 60 grams of the powdered stem of the plant is taken for extraction. The extract wasevaporated so, the solid extract obtained was 8.958 grams. Different fractions of the ethanolic extracts of the stem of *Scheonoplectus triqueter (L) palla* are evaluated for the antibacterial activity against two types of bacteria i.e. *staphylococcus aureus* and *Escherichia coli.*

The fractions of ethanolic extracts were taken in n-hexane, water, DCM and ethyl acetate through fractionation method. The results of the antibacterial activity show that n-hexane fraction is relatively an active fraction for the anti-bacterial activity of *E.coli* (gram negative) while the least active fractions wereDCM and water against *E.coli*.

On the other hand for *staphylococcus aureus* (gram positive) the relatively most active fraction was ethyl acetate showing 15 mm zone of inhibition while the least active fractions were DCM and water.

Table 1. Mean of the inhibitory zones for different extracts and standards against *E. coli* and *S. aureus*.

Strains used	*hexane*	*DCM*	*Ethyl acetate*	*water*	*clindamycin*	*ampicillin*	*kanamycin*
Escherichia coli	*19mm*	*8mm*	*10mm*	*8mm*	*20mm*	*15mm*	*26mm*
S. aureous	*11mm*	*8mm*	*15mm*	*8mm*	*20mm*	*15mm*	*26mm*

4. CONCLUSIONS

The *Scheonoplectus triqueter (L) palla* showing intermediate antibacterial activity in contrast to gram negative as well as against gram positive. The most significant antibacterial agent is absent in the proceeding ofthis plant as an antibacterial agent. This plant posses very low potential towards antibacterial activity.. Since no antibacterial activity was reported on the *Scheonoplectus triqueter (L) palla.* So it's important to do not proceeds their extracts for further purification and characterization

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