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# Phytochemical, Phytotoxic and Brine-shrimp lethality investigations of *Gazania rigens* L.

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*Gazania regins* is a native South African Plant, herbaceous perennial treasure flower belongs to *Asteraceae* family and is similar appearance to genus Arctotis.The fresh extract of plant is used for the treatment of various biological activities. In the present study, the phytochemical analysis indicated the presence of flavonoids, phenolic acids, alkaloids and glycosides in the plant. Thin layer chromatography of both extract is given the separation of different components. Dichloromethane extract (GRWPD) and methanol crude extracts (GRWPM)of plant were screened for and phytotoxic, antibacterial and brine shrimp lethality bioassay. Both extracts of *Gazania regins* inhibited the growth of *Lemna minor* L. and showed significant phytotoxic activities. The plant exhibited non-significant activity when tested against pathogenic gram positive and gram negative bacteria. Brine shrimp assay showed LD50(µg/ml) of methanolic extract 10678.37 and dichloromethane extract 20682.9 respectively. The current study on plant extracts suggested that *Gazania rigens* has a higher potential for phytotoxicity.

**Keywords:** ***Gazania rigens*, Antibacterial, Phyto-toxicity, Cytotoxicity, percentage yield**

## INTRODUCTION

The plants are being used to treat diseases and injuries from thousand years but also included much misinformation and superstition (El Manawaty et al. 2013). Due to diverse medicinal potential, every civilization has accumulated experience and knowledge of their use of plants. Western medicine system was centred in Egypt and Mesopotamia, while the Unani (Islamic) and Ayurvedic (Hindu) systems were originated in western Asia, the Indian subcontinent and those of the Orient from China, Tibet and Japan (Chaudhry and singh, 2016; Alves et al. 2012).

Worldwide most human beings died of bacterial infections (Zhang et al. 2016). Different species of bacteria belonging to two major classes, i.e. Gram negative like Pseudomonas and Salmonella and Gram positive Staphylococcus and Bacillus are responsible for severe infections because of their survival potential in harsh environment (Ahameethunisa and Hoper, 2010). Moreover, only one third of chronic diseases are treated from these synthetic products due to resistance (Sen and Batra, 2012), and these antibiotics may have serious side effects like hypersensitivity and immune suppression. That is the reason that discovery of new antibiotics is necessary and exploration for bioactive compounds may be very helpful in this discovery in treating resistant pathogenic microbes (Freire-Moran et al. 2011).

Today natural products are majr sources of new bioactive molecules. They may also serve as a model for new antibacterial drugs. Natural products may be obtained from such natural sources as animals, bacteria, fungi and plants (Chaudhry and singh, 2016; Alves et al. 2012).

Gazania regins is a half-hardy perennial native South African Plant, generic name Gazania came from the Greek ‘Gaza’ which refers to the rich color, variety and availability of the plant. The Afrikaans common name botterblom (butter flower) refers to its butter like taste when chewed. This herbaceous perennial treasure flower

belongs to Asteraceae family and is similar appearance to genus Arctotis. It is the native plant of South Africa (Beentje, 2010).The genus Gazania consists of 17 species & is related to the family Asteraceae with its subfamily Cichorioideae and tribe Arctotideae. Traditionally, Gazania splendens Moore is used to relieve toothache, earache, for stricture of urethra and to protect from miscarriage. Gazania krebsiana is regarded as soil stabilizers and also used as border plant. The infusion from roots of Gazania rigens variance uniflora was traditionally used by young Zulu men in magical sense when courting (Reddy et al. 2014).

Given the constant need to discover new plants and implicitly new sources of secondary metabolites with therapeutic potential that could be used in the treatment of inflammatory diseases and cancer the current attentions has been focused on an ornamental plant that is widespread and in certain classes of compounds such as polyphenols have already been identified, which may indicate therapeutic potential(Kane and Bhandari,2013; Bhalodia and Shukla 2011). Therefore, a study was designed to evaluate the methanolic and dichloromethane extracts of Gazania rigens for phytochemical and biological potential.

## MATERIALS AND METHODS

### Plant Collection and identification of plant

The plant was collected in the dry area of KotMithan, Rajanpur District (Pakistan). The plant was identified, and a voucherNo: IPBZU 378 specimen was issued by Institute of Pure and Applied Biology, BahauddinZakariya University, Multan, Pakistan.

### Preparation of plant extracts

The entire Gazania rigens plant was dried for 15 days under shade. After drying, plant material was grinded in a mill and weighed.A simple maceration method was adopted for the extraction of Gazania reigns. The grinded plant material (200gm) was added in an extraction bottle with a measured amount of solvent; dichloromethane. To achieve the best possible extraction, this mixture was stirred for a time and then homogenized in an ultrasonic bath. This mixture was filtered after 24 hours. Then, marc was macerated one again using the same procedure that was previously described. After the third collection of this extract, the marc was similarly extracted with methanol. The methanol and dichloromethane extracts were concentrated separately by Rotary evaporator, then weighed and given the codes; GRWPM & GRWPD respectively.

### Preliminary phytochemical analysis

The preliminary phytochemical analysis of whole herb was carried out according to the standard procedures (Ajayi et al. 2011).

### Thin layer chromatography (TLC) analysis

The dichloromethane and methanol extracts of Gazania rigens were subjected to TLC analysis by using suitable mobile phases. After development of TLC, Rf values of resolved components were calculated.

### Phytotoxic activity

Lemna bioassay was performed for phytotoxicity of GRWPM & GRWPD against growth of Lemna minor for search of new weedicides. Lemna minor is a phytotoxicity assay primarily used for screening in weedicide/herbicide search. The selective phytotoxicity of plant extracts can also be useful to develop new herbicide. The assay was run for 7 days and the rosset was counted on the 3rd or 7th day (Atta-ur-Rahman and Choudhary, 2001).

### Antimicrobial activity

Agar well diffusion method is widely used method to evaluate antibacterial potential of plant extracts (Bibi et al. 2010). Antimicrobial activity was recorded by measuring the zones of inhibition around the wells. All experiments were performed with fiver eplicates. % age growth inhibition was calculated by

$$\text{Inhibiton } (\%) = \left(\frac{\text{TS} - \text{SC}}{\text{PC}}\right) \times 100$$

where TS: test sample, SC: solvent control and PC : positive control

### Brine-shrimp lethality assay

The artificial seawater was prepared by adding 3.8g of NaCl in one litre H2O, filtered and then eggs of shrimps were added. After hatching, larvae were matured within two days at 22-29° temperature. The vials were prepared at 10, 100, 1000µl/mL concentrations for test. Each fraction was made in triplicate and 20mg plant extract was added in 2mL solvent. The sample was transferred in 5, 50, 500µL concentrations to vials corresponding to 10, 100, 1000µl/mL respectively. Etoposide was taken as a standard drug. In each vial, 10 shrimps and 5mL sea water(30 shrimps/dilution) were added and taken under illuminating light for 24h. The survived shrimps were numbered and used for Probit analysis (Finney Computer program) (Kadam et al. 2012).

## RESULTS

### Extract's Yield

The%yield of the plant extracts obtainedfrom the whole plant of Gazania rigens showed maximum in dichloromethane and least in methanolextract presented in Table 1.

**Table 1: %Yield of *Gazania rigens* extracts.**

| Plant name | Extract | Percentage yield (%) |
|---|---|---|
| *Gazania rigens* | Dichloromethane (GRWPD) | 15.5% |
| | Methanol (GRWPM) | 10.3% |

**Phytochemical tests**

The presences of secondary metabolites were evaluated by using In-vitro qualitative phytochemical study on the plant extracts and results were shown in Table 2.

**TLC analysis**

The dichloromethane plant extract was resolved into 12 components. The methanol plant extract was resolved into 4 components. Results are represented into Figure 1a and 1b. Stationary phase: Silica gel G60 F254, Mobile phase for (GRWPD) n-hexane : ethyl acetate (75:25) and for (GRWPM) Chloroform : methanol : water (80:20:02).

**(GRWPD) (GRWPM)**

**Phytotoxic activity**

The results of phytotoxic bioassay of crude methanol and dichloromethane extracts of Gazania rigens are given in the Table No 3.

**Table 2: The Phytochemical constituents of *Gazania rigens*.**

| Phytochemicals | Test name | G. rigens | |
|---|---|---|---|
| | | Dichloromethane | Methanol |
| *Alkaloids* | *Mayer's* | + | + |
| | *Dragendorff* | ++ | + |
| | *Wagner's* | + | + |
| | *Hager's* | + | + |
| *Proteins* | *Biuret* | - | - |
| | *Ninhydrin* | - | - |
| | *Millon's* | + | + |
| *Carbohydrates* | *Molish's* | + | + |
| | *Fehling's* | ++ | - |
| | *Barfoed's* | + | + |
| *Glycosides* | *Legal's* | + | + |
| | *Borntrager's* | + | + |
| | *Alkaline reagent* | + | ++ |
| *Tannins* | *Ferric chloride* | + | - |
| | *Gelatin* | + | - |
| *Flavonoids* | *Lead acetate* | + | ++ |
| *Saponins* | *Froth test* | - | + |
| *Sterols* | *Libermann-Burchard* | + | - |
| *Fixed oil* | *Spot* | - | + |
| | *Saponification* | - | ++ |

*"++" Strongly present "+" Indicates present & "–" Indicates absent*

**Table 3: *In-vitro* phytotoxic bioassay of *Gazania rigens***

| Extract of Plant | Plant Name | Conc. Of Compound (µg/ml) | No. of Fronds | % Growth Regulation | Standard Drug (Paraquat) Concentration (µg/ml) | |
|---|---|---|---|---|---|---|
| | | | Sample | Control | | |
| Methanol | Lemna minor | 1000 | 10 | 20 | 50 | 0.015 |
| | | 100 | 13 | | 35 | |
| | | 10 | 19 | | 5 | |
| Dichloromethane | | 1000 | 18 | | 10 | |
| | | 100 | 19 | | 5 | |
| | | 10 | 19 | | 5 | |

**Table 4: In vitro antibacterial bioassay of *Gazania rigens***

| Extract | Name of bacteria | Zone of inhibition of sample (mm) | Zone of inhibition of standard drug Imipenum (mm) |
|---|---|---|---|
| GRWPM | Eschericha coli | - | 25 |
| | Bacillus subtilis | - | 50 |
| | Shigellaflexinari | - | 28 |
| | Staphylococcus aureus | - | 48 |
| | Pseudomonas aeruginosa | - | 23 |
| | Salmonella typhi | - | 28 |
| GRWPD | Eschericha coli | - | 25 |
| | Bacillus subtilis | - | 50 |
| | Shigellaflexinari | - | 28 |
| | Staphylococcus aureus | - | 48 |
| | Pseudomonas aeruginosa | - | 23 |
| | Salmonella typhi | - | 28 |

**Table 5: Brime-Shrimp Lethality bioassay of *Gazania rigens***

| Extract | Dose (µg/ml) | No. of shrimps | No of Survivors | LD50 (µg/ml) | Standard Drug | LD50 (µg/ml) |
|---|---|---|---|---|---|---|
| Methanol | 1000 | 30 | 22 | 10678.37 | Etoposide | 7.4625 |
| | 100 | 30 | 25 | | | |
| | 10 | 30 | 29 | | | |
| Dichloromethane | 1000 | 30 | 23 | 20682.9 | | |

**In vitro antibacterial bioassay**

Sample of extracts (GRWPD & GRWPM) were tested against *Eschericha* coli, *Shigellaflexinari, Bacillus subtilis, Salmonella typhi*, Staphylococcus aureus and Pseudomonas aeruginosa by using Agar tube diffusion method. Both the extracts showed no antibacterial activity. Results are given in table No.4.

**Brine-shrimp lethality assay**

The results of phytotoxic bioassay of crude methanol and dichloromethane extracts of Gazania rigens are given in the Table No 5.

**DISCUSSION**

Two solvents like dichloromethane of intermediate polarity & highly polar; methanol were used to extract the whole plant of Gazania rigens. The percentage yield resulted from the dichloromethane is slightly higher than methanol. This yield difference of *Gazania rigens* extracts is associated to the compatibility of components with appropriate polarity of the solvent (Reddy et al. 2014). Because the dichloromethane is a solvent of intermediate polarity so it may extract out the components which are non-polar & slightly polar.

The therapeutic activities of the plants are mainly exhibited due the presence of phytochemicals such as; glycosides, phenols, saponins, flavonoids, carbohydrates, alkaloids and proteins. Alkaloids found in natural plants, mostly allied to the class of CNS, diuretics, analgesics, antimicrobial and antispasmodics (Kaur & Arora, 2015). Glycosides effectively control CVS related diseases. Saponins are commonly endorsed as anti-cancer related compounds (Yadav & Agarwala, 2011). In addition, glycosides and carbohydrates have beneficial roles in food supplements and strengthening immune system

(Balandrin et al. 1985; Stephane & Jules, 2020). The anti-oxidant potential is generally perceived in plants containing phenolic compounds like; tocopherols, phenolic acids and flavonoids (Ali et al. 2008). Anti-inflammatory, anti-carcinogenic, anti- aging, anti-apoptosis and cardio protective properties also found with phenolic constituents (Maleki et al. 2019; Eghbaliferiz & Iranshahi, 2016).

TLC of dichloromethane extract gave components with Rf values 0.301, 0.397, 0.438, 0.561, 0.643 and 0.712 appeared as dark spots under UV light at 254nm. Whereas the component with Rf values 0.287, 0.410, 0.561, 0.616, 0.863 and 0.931 showed fluorescence under UV light at 366nm. Some colored components became visible with Rf values 0.287 (Light green), 0.410 (Light green), 0.506 (Light purple), 0.561 (Light green), 0.684 (Purple), 0.767 (Light purple), 0.883 (Light green) and 0.931 (Purple) as shown in figure 1a. The methanolic extracts with components of Rf values 0.0137 and 0.917 appeared as dark spot under UV light at 254nm. While the components with Rf values 0.424 and 0.931 showed fluorescence under UV light at 366nm. Some colored components became visible as a L.P (Light purple) with Rf values respectively 0.671 and 0.931 after Godin reagent and 10% sulfuric acid spray as shown in figure 1b. According to Blackadar, 2016, cancer is the biggest cause of mortality globally. Numerous strategies have been developed to lessen the hazard that cancer poses. 60 percent of novel chemicals that were released between 1981 and 2002 are thought to have been natural products or derived from naturally occurring lead compounds, according to Nirmala et al. 2011. The most dependable, straightforward, and affordable cytotoxicity screening method that is connected with cytotoxicity and antitumor characteristics is the brine shrimp lethality bioassay. Weeds are one of the major causes of poor agricultural productivity in Pakistan. So there is a need to discover new natural weedicides (herbicides) because synthetic weedicides available in the market are expensive, toxic and non-specific. The Lemna minor assay is a quick measure of phytotoxicity of plants extracts.

Although synthetic herbicidal chemicals are primarily employed to manage weeds, their excessive use has raised concerns about environmental contamination and herbicide resistance. Alternative weed management methods that are both economical and environmentally benign are urgently needed everywhere. These growth inhibitors are included in phytotoxic plants in order to address the issue brought on by synthetic herbicidal pesticides (Hulzebos et al. 1993). The current study on plant extracts suggested that *Gazania rigens*has a higher potential for phytotoxicity. To identify and describe the bio-herbicides in this plant, more investigation is needed.

## CONCLUSION

The present findings have clearly demonstrated that the plant has excellent potential towards the phytotoxic activity. The growth inhibition assay of the plant showed least antimicrobial activity. Thin layer chromatography also showed the preliminary images of different components present in this plant. So the further studies are needed to purify the active constituents of the plants that are responsible for its phytotoxic.

## CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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## AUTHOR CONTRIBUTIONS

SA wrote the manuscript and performed the experimental work, SI rechecked the manuscript and designed the experimental work. NA helped in anatomical study and performance of experimental work, SR assisted throughout the experimental as well as theoretical work.



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