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Bio Evaluation of Different Fractions of *Gazania rigens*

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This communication describes the *in vitro* anti-bacterial, anti-fungal and anti-oxidative evaluation results of the different extracts of *Gazania* (Asteraceae). Whole plant was extracted using distilled n-hexane, DCM, ethyl acetate methanol and methanol: water subsequently. These fractions were further tested for *in vitro* anti-bacterial, anti-fungal and anti-oxidative activities using standard methods like micro broth dilution method for anti-bacterial and fungal, DPPH radical scavenging and H_2O_2 scavenging activity methods for anti-oxidative activity. The investigations resulted fractions with good anti-bacterial, anti-fungal and anti-oxidative activities, results were tabulated. Based on the results it was concluded that DCM, ethyl acetate, methanol and methanol: water fractions have good bio activity, these fractions may be further investigated for the pure compounds.

Key words*:* *Gazania*, micro broth dilution, DPPH radical scavenging activity, H_2O_2 scavenging activity.

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 and prefers to grow in sandy to average well drained soil-in full sun but needs consistent moisture and it is a good soil protector. It has tender perennial decumbent stems that spread along the ground, Silvery green leaves and daisy like flower heads which are usually bright orange with a black base. Flowering takes place during most of the year and seeds are egg shaped and covered with long hair. In Australia, where it is known as coastal Gazania, the species has become naturalized on coastal dunes and roadsides in the Central Coast and Sydney regions of New South Wales as well as the coast of South East Queensland. In South Australia it is found in the southern Mount Lofty area as well as on the Eyre Peninsula. There are three currently recognized varieties[1] (African plants database, 2008). *G.rigens* (L.) Gaertn. Var. *leucolaena* (DC.) Roessler. In cultivation, this variety is referred to as Trailing Gazania. *G.rigens* (L.) Gaertn. Var. *rigens* this variety is only found in cultivation, where it is known as Clumping Gazania. It is distinguished by its large 4 to 8 cm flower heads with yellow or orange rays, each with a basal eye-spot[2] (Aluka , 2008). *G. rigens* (L.) Gaertn. var. *uniflora* (L.f.) Roessler. Overly moist soils can lead to root and stem rot. Watch for leaf spot and powdery mildew

Edible Uses

Gazania rigens (Asteraceae) is the most common *Gazanias* species sold in the market today. *Gazania krebsiana subSp. Serrulata*'s (Asteraceae) flower is reported to have edible uses (Ref). *Gazanis linearis* known as ornamental one has been described as having unusual uses such as[3-4] (Hutchinson, J. (1934). *Gazania linearis*. Curtis's Bot. Mag. Beentje**, H.J.** 2010).

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Medicinal Uses

The flowers of *Gazania krebsiana* subSp. *Serrulata*'s(Asteraceae) is reported to be useful in treating earache in babies and are also used for treating sterility[5] (Swazilands Flora Database). *Gazania* species have reported a range of medicinal uses as well being used as possible food source with health benefits. These results prompted the present study whose aim is to investigate the *in vitro* anti-bacterial, anti-fungal and anti-oxidative activities of hexane, dichloromethane (DCM), methanol and methanol: water fractions of whole plant extraction of *Gazania rigens*.

MATERIALS AND METHODS

The plant *Gazania rigens* (Asteraceae) is collected from silver glen nature Reserve in Durban South Africa, plant was identified by Prof.Ashley Nicholasin, School of Life Sciences at University of Kwazulu-Natal,Westvelli Campus in Durban, South Africa. Voucher specimen was deposited (No-3681).The whole plant of *Gazania* rigens (Asteraceae) was grounded and extracted using distilled n-hexane, DCM, methanol and methanol: water subsequently.

Microbial strains

Gazania rigens (Asteraceae) plant extracts were tested on five bacteria and four yeast strains of *Candida*. For the antibacterial assay, American Type Culture Collection (ATCC), *Escherichia coli* (35218), *Staphylococcus aureus* (43300), *Entero faecalis* (5129), *Klebsialla pneumoniae* (700603), *and Pseudomonas aeruginosa* (27853) strains were used. The bacterial strains were cultured on Muller-Hinton agar plates, incubated at 37°C for 24 h.

Four yeast strains of *Candida* from American Type Culture Collection, *Candida albicans* (90028), *Candida albicans* (10231), *Candida krusei* (6258) and *Candida parapsilosis* (22019) were used. Yeast strains were grown on Sabouraud dextrose agar, incubated at 35°C for 24 h and used for antifungal assay.

Antimicrobial assay

Antifungal/antibacterial agents: Plant extracts were dissolved in DMSO and the final concentration in each well was made to 10% DMSO. All dilutions were freshly prepared before application. Amphotericin-B and Neomycin from Sigma Aldrich were used as reference drugs for antifungal and antibacterial assays respectively. The final concentration of antifungal and antibacterial test solutions ranged from 0.0012 to 200 µg $mL^{-1,}$ amphotericin-B 0.0015 to 100µg mL^{-1} and neomycin 0.0076 to 500µg mL^{-1}. All dilutions were carried out in 96-well flat bottom microtitre plates.

Antifungal susceptibility test

Evaluation of the susceptibility of *Candida albicans* and non- *Candida albicans* species were performed using the broth micro dilution method according to M27-A2 for yeast guidelines[6] (NCCLS, 2002). Yeast strains were grown aerobically overnight at 35 °C on Sabouraud dextrose agar plates. Yeasts were harvested and suspended in 1% sterile saline and the turbidity of the supernatants were measured spectrophotmetrically at 625 nm with an absorbance of 0.08-0.1 equivalents to the 0.5 Mc Farland standard following the NCCLS M27-A2 guidelines. The working suspension was diluted 1:20 in a mixture containing RPMI 1640 medium with and 0.165M morpholinepropanesulfonic acid buffered to pH 7.0. The working suspension was further diluted with the medium (1:50) to obtain the final test inoculums ($1\text{-}5x10^{3}$CFU ml^{-1}). The microtitre plates were allowed to thaw and equilibrate to room temperature under aseptic conditions which contained different concentrations of test solutions. Aliquots of working inocula suspensions were dispensed into each well and the plates were incubated in an aerobic environment at 35 °C for 24 h. After incubation, 20 µl of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-18sulfophenyl)-2H-terazoliumsalt (MTS,Promega Corporation, Madison, USA) was added directly to each well, incubated at 37ºC for 4 h and the absorbance recorded at 490 nm on a 96-well plate reader (VACUTEC). All analyses were performed in triplicate and data are reported as the mean ± standard error of the mean of ≤ 5.

Antibacterial susceptibility test

The bacterial susceptibility test was carried out using micro broth dilution method[7] (Schwalbe, R, 1998). Overnight cultures (16-18 h of incubation at 37ºC) were adjusted to a turbidity of 0.5 McFarland standards. Inocula were adjusted to 0.08-0.1 to yield a stock suspension of $0.4\text{-}5x10^{8}$

CFU mL^{-1} which was diluted one hundred fold to obtain a working suspension of 10^6 CFU mL^{-1} at 625 nm. Microtitre plates were placed in a laminar flow unit to equilibrate to room temperature under aseptic conditions. Aliquots of 100 µL of bacterial inocula were added to the micro titer plates containing different concentrations of test solutions. The plates were incubated aerobically for 16-18 h at 37 °C. Following incubation, added 40µL of freshly prepared iodonitrotetrazoliumchloride 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H tetrazolium chloride, INT) solution (200µg mL^{-1}) was added to each well and the plates were further incubated for 45 minutes at 37ºC in the dark. If the colorless INT is reduced to red after incubation; persistent growth of bacteria is indicated. No color change denotes the lack of bacterial growth. Neomycin was used a control drug in this study. All analyses were performed in triplicates and the data are reported as the mean ± standard error of the mean of ≥ 5.

In vitro antioxidant activity

DPPH radical scavenging activity

Free radical scavenging activity was determined by using 2, 2'-diphenly-1-picrylhydrazyl (DPPH) method followed by Burits and Bucar[8] (Burits M, 2000). One mL of various concentrations of the extracts in methanol was added to 4mL of 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical by DPPH in percent (I %) was calculated by using the following equation.

I % = [(A control – A sample) / A blank] x 100.

Where A control is the absorbance of the control reaction (containing all reagents except the test solution), and A sample is the absorbance of the test solution.

H_2O_2 scavenging activity

The H_2O_2 scavenging activity of plant extracts was determined according to the method described elsewhere[9] (Serhat Keser., *et al*, 2012). A solution of H_2O_2 (40mM) was prepared in phosphate buffer (pH 7.4). Different concentrations of plant extracts in 3.4 mL phosphate buffer were added to a H_2O_2 solution (0.6mL, 40mM). The absorbance value of the reaction mixture was recorded at 230 nm. The % of inhibition was calculated.

RESULTS AND DISCUSSION

Table one shows anti-bacterial assay results, among all the fractions dichloromethane fraction, ethyl acetate fraction and methanol fraction showed good activity. Table two shows anti-fungal activity results of all the fractions, dichloromethane and methanol: water fractions showed good activity. Table three shows DDPH radical scavenging activity results for anti-oxidative activity, Ethyl acetate fraction showed strong activity compared to all fractions at all concentrations and remaining fractions showed good activity. Table shows H_2O_2 scavenging activity results for anti-oxidative activity, among all the fractions dichloromethane, ethyl acetate, methanol and methanol: water fractions showed strong activity.

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