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Short Communication [Kısa Bildiri]

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Bio evaluation of different crude extracts of *Chrysanthimoides monilifera* subsp.*rotundata*

[*Chrysanthimoides monilifera* subsp.*rotundata* farklı ham özütlerinin biyodeğerlendirilmesi]*

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ABSTRACT

Aim: This communication describes the *in vitro* anti-bacterial, anti-fungal and anti-oxidative evaluation results of the different extracts of leaves, stem and roots of *Chrysanthimoides monilifera* subsp. *rotundata* (Asteraceae) for the first time.

Materials and Method: Leaves, stem and roots of the plant were used for extraction by using distilled n-hexane, DCM, ethyl acetate and methanol. These fractions were tested for their *in vitro* anti-bacterial, anti-fungal and anti-oxidative activities using standard protocols like micro broth dilution method for anti-bacterial and anti-fungal, DPPH radical scavenging and H_2O_2 scavenging activity methods for anti-oxidative activity.

Results: The investigations resulted fractions with good anti-bacterial, anti-fungal and anti-oxidative activities which were tabulated.

Conclusions: Based on the results it was concluded that these fractions with good bioactivity may be further researched for the pure compounds.

Key Words: *Chrysanthimoides monilifera* Subsp. *rotundata*, bioactivity, DPPH radical scavenging, H_2O_2 scavenging.

Conflict of Interest: Authors have no conflict of interest.

ÖZET

Amaç: Bu kısa bildiride, ilk defa olarak**,** *Chrysanthimoides monilifera* subsp. *rotundata* (Asteraceae)'nın yaprak, kök ve gövde ekstrelerinin anti-bakteriyal, anti-fungal ve anti-oksidatif özellikleri incelenmiştir.

Gereç ve Yöntemler: Yaprak, kök ve gövde ektraksiyonları n-heksan, DCM, etil asetat ve methanol distilasyonu ile yapılmıştır. Bu fraksiyonların *in vitro* anti-bakteriyal, anti-fungal ve anti-oksidatif aktiviteleri, standart protokoller kullanılarak test edilmiştir: anti-bakteriyal için mikto büyüme dilüsyon metodu; anti-fungal için DPPH radikal oluşum metodu; anti-oksidatif aktivite için H_2O_2 oluşum ve koruma metodu.

Bulgular: İyi anti-bakteriyal, anti-fungal ve anti-oksidatif aktivite araştırma sonuçları tablolandırılmıştır.

Sonuç: Sonuçlara dayalı olarak fraksiyonlrın güçlü biyoaktiviteleri olduğuna karar verilmiştir ve ileride saf bileşiklerle çalışmalar yapılması planlanmaktadır.

Anahtar Kelimeler: *Chrysanthimoides monilifera* Subsp. *rotundata*, biyoaktivite, DPPH radikal oluşum, H_2O_2 oluşumu.

Çıkar Çatışması: Yazarlar çıkar çatışması bulunmadığını beyan ederler.

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Introduction

Chrysanthimoides monilifera subsp *rotundata* (*C. m. rotundata*) is an enduring shrub belonging to Asteraceae family. It has densely spread branches usually growing up to 1-2 meters tall and 2-6 meters across and prefers warm climates. The stem of this plant has many branches which are green or purplish green when young. They become woody with age, especially towards the base of the plant. The leaves are petioles and are arranged alternatively, 8-25 mm long, and be slightly fleshy in nature. The flower-heads are daisy-like with bright yellow petals 8-13 mm long and are borne in small clusters at the tips of branches and usually have more than ten petals. Although flowering occurs throughout the year it happens mostly during the cooler late autumn and winter months. The fleshy fruits are green when young but as they mature their skins turn blackish.

Edible Uses

Some of the *Chrysanthemoides* species are edible, *Chrysanthemoides monilifera* is formerly used by the Khoi and san tribes of South Africa as a food source [1]. Tea made from the flowers of *Chrysanthemum indicum* is very popular in East Asia.

Medicinal Uses

Some *Chrysanthemum* species have good medicinal values i.e. tea made from the *C. indicum* flowers is reported to have health benefits such as detoxification of blood, sinus congestion and for high blood pressure. The tea made from flowers of *C. indicum* inhibits the growth of bacteria in the body such as *Staphylococcus aureus*, *Sterptococcus hemolyticusb*, *Psedomonas aeruginosa*, *Shigella dysenteriae*, *tubercle bacillis* and *dermatomycosis*. This tea concoction is said to ease the digestion and is also believed to improve vision and hearing. In Chinese traditional medicine tea made from *C. indicum* is used to treat some eye diseases [2]. The buds of *C. indicum* has long history in Chinese traditional medicine mainly for treating inflammation, hypertensive symptoms, respiratory diseases, prevention of thrombosis and has antibacterial, antifungal and antiviral activity [3-4]. The ethanol extract of *C. indicum* reported to inhibit to invade hepatocellular carcinoma and studies on *Chrysanthemum cinerariaefolium* and *Chrysanthemum coccineum* showed the presence of pyrethroids which are used as insecticides [5]. Essential oils and chemical constituents from *C. indicum* reported to have good anti-bacterial activity against oral bacteria [6-10]. These reported medicinal and edible uses of genus *Chrysanthemum* prompted the present study whose aim was to investigate the *in vitro* anti-bacterial, anti-fungal and anti-oxidative of hexane, dichloromethane (DCM) and methanol fractions of stem, roots and leaves of *C. m. rotundata*.

Materials and Methods

The plant *C. m. rotundata* (Asteraceae family) was collected from Silver glen Nature Reserve in Durban, South Africa. The plant was identified in the School of Life Sciences, University of KwaZulu-Natal, Westville Campus in Durban, South Africa, voucher specimen was deposited (No. 9411).

The plant was dried in drying room for several days, then the leaves stem and roots of the said plant were separated from the plant. These parts with ground separately and extracted using different solvents like n-hexane, DCM and methanol later.

Microbial strains

C. m. rotundata plant extracts were tested on five bacteria and four yeast strains of *Candida*. For the anti-bacterial test, American Type Culture Collection (ATCC) strains, *Escherichia coli* (35218), *Staphylococcus aureus* (43300), *Enterococcus faecalis* (5129), *Klebsialla pneumoniae* (700603), *and Pseudomonas aeruginosa* (27853) 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 test.

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 [11]. 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, MTS 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. Amphotericin-B was used as reference drug.

Antibacterial susceptibility test

The bacterial susceptibility test was carried out using micro broth dilution method [12]. Overnight cultures (16-18hrs of incubation at 37 C) were adjusted to a turbidity of 0.5 McFarland standards. Inocula were adjusted to 0.08-0.1 to yield a stock suspension of 0.4-5x10^8 CFU mL^{-1} which was diluted one hundred fold to get a working suspension of 10^6 CFU mL^{-1}at 625 nm. Microtiter plates were placed in a laminar flow unit to equilibrate to room temperature under aseptic conditions. Aliquots of 100µL of bacterial inocula were added to the micro titer plates containing different concentrations of test solutions. The plates were incubated aerobically for 16-18hrs at 37°C. Following incubation, 40µL of freshly prepared INT solution (200µg mL^{-1}) was added to each well and the plates were further incubated for 45 minutes at 37ºC in the dark. Reduction of INT to red color indicates persistent growth of bacteria; no color change indicates 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

Free radical scavenging activity was determined by using 2, 2'-diphenly-1-picrylhydrazyl (DPPH) method prescribed elsewhere [13]. One ml of various concentrations of the extracts in methanol was added to 4mL of 0.004% methanol solution of DPPH. After a 30min 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. Ascorbic acid was used as reference.

$I\ \% = [(A_{control} - A_{sample}) / A\ blank] \times 100.$

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

The H_2O_2 scavenging activity of plant extracts was determined according to the method described elsewhere [14]. 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. Ascorbic acid is used as control for both types.

Results and Discussions

Table 1 shows results of anti-bacterial activity of crude fractions of *C. m. rotundata*. The Bio evaluation of twelve fractions yielded good to comparative activity. Hexane and methanol fractions of *Chrysanthus* leaves, hexane fraction of *Chrysanthus* stem and roots showed good activity on *S. aureues* and *E. faecalis* which are gram positive. DCM and ethyl acetate fractions of leaves, DCM, ehtyl acetate, methanol fractions of stem and hexane, DCM, ethyl acetate and methanol fractions of *Chrysanthus* roots showed comparative activity. On gram negative bacteria, only hexane fraction of *Chrysanthus* leaves showed comparative activity, remaining fractions did not show any activity against gram negative bacteria.

Table 2 shows results of anti-fungal activity of crude fractions. Hexane DCM, ethyl acetate and methanol fractions of *Chrysanthus* leaves showed good activity against *C. albicans ATCC* 90028 and *C. albicans ATCC 10231,* ethyl acetate fraction also showed good activity against *C. krusei ATCC 6258* and *C. parapsilosis AECC 22019.* . Hexane fraction of *Chrysanthus* stem showed good activity against *C. albicans ATCC 90028*, *C. albicans ATCC 10231, C. krusei ATCC 6258* and *C. parapsilosis*. DCM and ethyl acetate fractions of *Chrysanthus* stem showed good activity against all the strains used, but methanol fraction of *Chrysanthus* stem did not show any activity against used strains. Hexane, DCM, ethyl acetate and methanol fractions of *Chrysanthus* roots showed good activity against all the strains used.

Anti-oxidative activity of the *C. m. rotundata* is seen in Table 3 and 4. Table 3 shows results of DPPH radical scavenging activity of crude fractions. Fractions resulted strong to good activity at the concentrations tested in this method. At 25%, methanol fraction of *Chrysanthus* roots showed strong activity, hexane, DCM, ethyl acetate and methanol fractions of leaves, hexane, ethyl acetate and methanol fractions of stem and hexane and ethyl acetate fractions *Chrysanthus* roots showed good activity. At 50%, hexane fractions of leaves, roots and methanol fractions of stem and roots showed strong activity, remaining fractions showed good to comparative activity. At 75%, 100% and 250%, hexane fraction of leaves, stem and roots, ethyl acetate fraction of leaves and methanol fraction of stem and roots showed strong activity, remaining fractions showed good to comparative activity.

H_2O_2 Scavenging activity of crude fractions of *C. m. rotundata* is shown in Table 4. Fractions showed strong to good activity at the concentrations tested in this method. Methanol fraction of stem, hexane, and DCM fraction of leaves and ethyl acetate and methanol fractions of *Chrysathus* roots showed strong activity at all the concentrations tested. All the remaining fractions showed good to comparative activity.

Conclusion

The extracts have good anti-bacterial, anti-fungal and anti-oxidative activities. These plant extracts may be further purified for the chemical components.

Table 1. Results of Minimum Inhibitory Concentration (MIC mg ml$^{-1)}$ of Chrysanthemum plant extracts of different solvent fractions on various bacterial strains.

S.No	*S.aureues* Gram+ve	*E.faecalis* Gram+ve	*E.coli* Gram-ve	*P.aeruginosa* Gram-ve	*K.pneumoniae* Gram-ve
Hexane fraction of Chrysanthus Leaves	0.25	0.25	1	1	-
DCM fraction of Chrysanthus Leaves-1	0.5	0.75	-	-	-
Ethyl acetate fraction of Chrysanthus Leaves	1	0.75	-	-	-
Methanol fraction of Chrysanthus Leaves	0.5	0.75	-	-	-
Hexane fraction of Chrysanthus Stem	0.25	0.25	-	-	-
DCM fraction of Chrysanthus Stem	0.75	0.75	-	-	-
Ehyl acetate fraction of Chrysanthus Stem	0.5	0.75	-	-	-
Methanol fraction of Chrysanthus Stem	0.75	0.75	-	-	-
Hexane fraction of Chrysanthus Roots	0.5	0.5	-	-	-
DCM fraction of Chrysanthus Roots	1	1			
Ethyl acetate fraction of Chrysanthus roots	1	0.75	-	-	-
Methanol fraction of Chrysanthus roots	0.125	0.125	-	-	-
Control (μg mL^{-1})	10	20	20	10	12

All experiments were carried out in triplicate. Data reported as the mean±standard error of the mean ≤5. .“-“ve indicates No activity. Control indicates Neomycin.

Table 2. Results of Minimum Inhibitory Concentration (MIC mg ml$^{-1)}$ of Chrysanthemum plant extracts of different solvent fractions on various yeast strains.

S.No	*C.albicans ATCC 90028*	*C.albicans* ATCC*10231*	*C.krusei ATCC 6258*	*C.parapsilosis ATCC22019*
Hexane fraction of Chrysanthus Leaves	0.125	0.125	-	-
DCM fraction of Chrysanthus Leaves-1	0.5	0.5	-	0.75
Ethyl acetate fraction of Chrysanthus Leaves	0.5	0.5	1	1
Methanol fraction of Chrysanthus Leaves	0.125	0.125	-	1
Hexane fraction of Chrysanthus Stem	0.125	0.125	1	1
DCM fraction of Chrysanthus Stem	0.75	0.75	0.75	0.75
Ethyl acetate fraction of Chrysanthus Stem	0.5	0.5	0.25	0.25
Methanol fraction of Chrysanthus Stem	-	-	-	-
Hexane fraction of Chrysanthus Roots	0.5	0.75	0.75	0.75
DCM fraction of Chrysanthus Roots	0.125	0.5	0.75	0.75
Ethyl acetate fraction of Chrysanthus roots	-	1	0.75	0.75
Methanol fraction of Chrysanthus roots	1	1	-	-
Control (μg mL^{-1})	20	25	22	15

All experiments were carried out in triplicate. Data reported as the mean±standard error of the mean ≤5. .“-“ve indicates No activity. Control indicates Amphotericin-B.

Table 3. DPPH Radical scavenging activity (μgml^{-1})

S.No	Fraction Name	Concentration (%)				
		25	50	75	100	250
1	Hexane fraction of Chrysanthus Leaves	39	51	69	75	89
2	DCM fraction of Chrysanthus Leaves-1	30	45	68	72	84
3	Ethyl acetate fraction of Chrysanthus Leaves	32	56	69	79	89
4	Methanol fraction of Chrysanthus Leaves	25	45	59	65	74
5	Hexane fraction of Chrysanthus Stem	41	48	69	73	91
6	DCM fraction of Chrysanthus Stem	29	39	59	67	86
7	Ethyl acetate fraction of Chrysanthus Stem	41	49	52	61	69
8	Methanol fraction of Chrysanthus Stem	39	56	71	86	94
9	Hexane fraction of Chrysanthus Roots	42	58	69	81	87
10	DCM fraction of Chrysanthus Roots	18	25	36	45	54
11	Ethyl acetate fraction of Chrysanthus roots	33	45	59	69	78
12	Methanol fraction of Chrysanthus roots	45	62	78	84	91
13	Control-Ascorbic acid	40	52	68	87	98

Table 4. H_2O_2 Scavenging activity (μgml^{-1})

S.No	Fraction Name	Concentration(%)				
		25	50	75	100	250
1	Hexane fraction of Chrysanthus Leaves	49	61	79	84	91
2	DCM fraction of Chrysanthus Leaves	42	59	68	75	91
3	Ethyl acetate fraction of Chrysanthus Leaves	41	53	69	75	86
4	Methanol fraction of Chrysanthus Leaves	39	51	59	68	79
5	Hexane fraction of Chrysanthus Stem	49	58	69	78	84
6	DCM fraction of Chrysanthus Stem	48	66	78	83	89
7	Ethyl acetate fraction of Chrysanthus Stem	44	65	79	85	89
8	Methanol fraction of Chrysanthus Stem	56	71	85	91	94
9	Hexane fraction of Chrysanthus Roots	51	69	78	88	95
10	DCM fraction of Chrysanthus Roots	43	59	69	75	81
11	Ethyl acetate fraction of Chrysanthus roots	45	69	78	81	88
12	Methanol fraction of Chrysanthus roots	42	69	79	86	92
13	Control-Ascorbic acid	52	66	79	85	96

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