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Research Article

EXPLORATION OF BIOACTIVE SCREENING AGAINST THE MICROBIAL ORGANISMS FROM THE TWO DIFFERENT *CHRYSANTHEMUM* MEDICNAL PLANT FLOWER WITH TWO ASSORTED EXTRACTS

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ABSTRACT

Chrysanthemum plant is an ayurvedic important medicinal plant, and modern systems of medicine, flowers are the most important part in the field for preparation of various drugs. The present study aimed to focused objective was to study the two flower extracts with methanol and ethanol solvents and its antimicrobial activity both bacteria (*Staphylococcus* and *Pseudomonas* strains) and fungi (*Candida* species). When the *C. indicum* plant leaf extract treated with the experimental organisms of three strains *Pseudomonas* strains clearly showed that the maximum and minimum zone of inhibition was noticed on PA-38, 8±1.38 and PA- 37, 4±0.11. Similarly, ethanolic extract illustrated that the higher activity of this strain PA-39, 11±2.68 as well as PA-38, revealed the observed the value of 9±0.65 minimum effect on ethanolic extract. While, the *C. cinerariaefolium* ethanolic flower extract was treated with three different strains of *Pseudomonas* maximum similar zone of inhibition was noticed against PA-37 (10 ±2.75) and PA-38 (10 ±2.65). It was statistically significant as well as highest response when compared with Ciprofloxacin. Amoung the two plants *C. indicum* possessed excellent antimicrobial activity on both bacteria and fungi than the *C. cinerariaefolium* plant leaf. Subsequently the present research analyzed flower of the *C. indicum* plant by GC-MS techniques in order to determine the majority compounds. Totally 18 compounds were analyzed, amoung the 18 analytes camphor is an important peak compound also it contains 19.5 and 0.60% retention time and abundance respectively. Furthermore *C. indicum* possessed second most compound is Isoborneol, it was probably noticed 0.410% of abundance and its retention time is 17.5. Additionally two compounds also been observed as a sub peak level such as γ- Cadinene Methyl ester and Chrysanthenol. Hence, the current result clearly showed that the C. indicum plant flower is act as a very good potential antimicrobial agent.

KEYWORDS: *Chrysanthemum indicum* (Linn.) Ethanol, Methanol, antimicrobial activity

INTRODUCTION

Chrysanthemum (*Chrysanthemum indicum* L.) is a well-known Thai and Chinese herbal tea. The whole plant has health benefit but the famous part is the flower used in chrysanthemum tea [1]. *C. indicum* always used in traditional drug formula for the treatment of several

infectious disease such as pneumonia, colitis, stomatitis, cancer, fever, sore and used to treat vertigo, pertussis and hypertensive symptom [2]. Active compounds in *C. indicum* are glycosides, adenine, and flavanoids. Previous research work also showed that *C. indicum* has the ability to act as antibiotic to many species of bacteria [3].

Chrysanthemum indicum Linn is traditionally reported for the antiarthritic [4] anti-inflammatory and immunomodulatory [5] and hepatoprotective activity [6]. The plant extracts possess central and peripheral analgesic properties, lowering blood pressure, also exhibited inhibitory activity against microbes [7]. The tea prepared from *C. indicum* flower could prevent sore throat and promote reduction of fever. The flower extract have antioxidant activities and DNA damage preventive capacity [8]. So far, there is a very little work has been conducted by other researchers, since the current work was designed the following objectives such as (i) to determine the antimicrobial (Bacteria and Fungi) activity against *Pseudomonas* and *Staphylococcus* strain also *Candida* strain of the fungal species with three different solvent extract of the flower from *Chrysanthemum* plant ii) To demonstrate the bioactive compounds and elucidate the analytes of the ethanolic extract of the *C. indicum* flower through GCMS analysis.

MATERIAL AND METHODS

Collection of Plant Materials

Leaves of the both plants such as *Chrysanthemum indicum* and *C. cinerariaefolium* flowers were collected during the month of September, 2014 from Kanyakumari District of Tamilnadu, (India).

Preparation of Plant Extracts

The method of Mohan et *al.,* 2009 was followed for preparation of the extracts. Precisely, fresh plant leaves were washed under running tap water, soaked for 10 minutes in sterilized distilled water and then air dried. The dried leaves were ground to fine powder with the help of pestle and mortar. The plant powder was stored in air- tight bottles. Aqueous extract 20% w/v and alcoholic extracts 10% w/v were prepared for use in the study

Pathogens: From three strains of Bacterial pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* were obtained from High Ground Medical College, Anna nagar, Tirunelveli. Five isolates of *Candida* species, *Candida albicans* two stains and one strain each of *Candida tropicalis*, *C. parapsilosis* and *C. kruzei* were obtained from National Culture Collection of Pathogenic Fungi too gained from the similar place.

In vitro susceptibility of Bacterial and Fungal Pathogens to Plant Extracts

All the extracts of plants were tested for antimicrobial activity against different isolates of bacteria and fungi by Disc diffusion method.

Preparation of Inoculum

Inoculums were prepared by suspending 5-8 colonies of each organism from the fresh cultures in 5ml of normal saline in a test tube followed by vortexing. The turbidity of each suspension was compared with 0.5 Macfarland standards.

Antibacterial Assay

Diffusion method was followed for determining inhibitory activity of the plant extracts on the specified bacterial isolates using Muller Hinton Agar plates. The medium was sterilized by autoclaving at 121°C for 15 minutes, poured in petriplates, allowed to solidify at room temperature, incubated at 37°C overnight. The sterilized cotton swabs dipped in bacterial inoculum were swabbed over agar plates in order to spread the inoculum uniformly. After drying for five minutes, sterile discs dipped in 10µl of the plant extracts were placed on the surface of the inoculated medium and the extracts were then allowed to diffuse for 5 minutes. The plates were incubated at 37°C for 24-48 hours and observed for the inhibition of the growth around the discs thereafter. The antibacterial activity was determined by measuring the diameter of zones of inhibition of bacterial growth around the discs. Ampicillin (10mcg) and Gentamicin (10mcg) were also included in the assay against *Staphylococcus aureus* strains and Ciprofloxacin (5mcg) against *Pseudomonas aerugenosa* strains. The solvent controls were also kept to see if they had some inhibitory activity.

Antifungal Assay

In antifungal assay, Sabouraud's Dextrose Agar (SDA) medium was used for the growth of fungal strains. This medium was prepared in distilled water and sterilized by autoclaving at 121°C for 15 minutes; the medium was poured into sterile petriplates under aseptic conditions and allowed to solidify at room temperature. The sterile cotton swabs dipped in fungal inoculum were swabbed over the SDA plates and allowed to dry for 5 minutes. Sterile discs dipped in 10µl of the plant extracts were placed on the surface of the inoculated medium and the extracts allowed diffusing for 5 minutes. The plates were incubated at 28°C for 24-48 hours and observed for the inhibition of the growth around the discs. The antifungal activity was determined by measuring the diameter of zones of inhibition of fungal growth around the discs. Amphoterecin B (25mcg) was also used to see its antifungal activity. The respective solvents were also kept as controls to see if they had the some inhibitory activity.

GC-MS Analysis

Analytical GC was carried out on a varion 3300 GC fitted with a silicone DB-1 capillary column (30 m X0.25 mm), film thickness 0.25 µm, carrier gasnitrogen, flow rate 1.5 ml/min., split mode,temperature programmed 180º – 250ºC at 4ºC/min.Injector temperature and detector temperature were250ºC and 300ºC, respectively. Detector used wasFID. Injector volume for all samples was 0.1 µl.

GC-MS analysis

GC-MS analysis was carried out on a Schimadzu QP-2000 instrument at 70 eV and 250ºC. GC column Ublon HR-1

fused silica capillary 0.25 mm X 50 m with film thickness 0.25 µm. The initial temperature was 100ºC for 6 min and then heated at a rate of 10ºC/min. to 250ºC. Carrier gas, helium, flow rate 2 ml/min, detector used was FID.

Identification

The volatile components were identified by comparing their retention indices of GC chromatograph with those of literature. Further identification was done by GCMS. The fragmentation patterns of mass spectra were compared with those of the spectrometer data base using NBS 54 AL and Wiley L – built libraries.

RESULTS AND DISCUSSION

Table 1: *Bioactive spectrum of antibacterial activity of Methanolic and Ethanolic Extracts of Chrysanthemum cinerariaefolium (L.) Against the Staphylococcus Strains*

Test (*Staphylococcus* strains)	Methanolic extract (Diameter mm)	Ethanolic extract (mm)	Gentamicin 10mcg disc	Tendency with (Gentamicin)	Vulnerability to Ampicillin	Negative control (Solvent alone)
SPMIC-29	10 ±0.45**	9 ±1.54	6± 1.20	S	R	-
SPMIC-130	9 ±1.95	11 ±2.94*	12± 3.6**	S	R	-
SPMIC-132	11 ±1.76	6 ±0.84	9 ±1.65*	S	R	-

**- Significant at 5% level*

***- Highly significant at 0.01% level*

R = Resistant, S = Sensitive, - = No zone of inhibition around the disc

Initially Table 1 show that the antibacterial activity of leaf extracts with methanolic and ethanolic solvent of *C. cinerifolium.* Amoung the three different staphylococcus strain SPMIC-132 showed the significantly highest diameter of zone 11±2.64appeared on ehtanolic leaf extract, followed by similar zone of inhibition was noticed against SPMIC-130- 7±0.87 and SPMIC-29 7±0.54 on both experimental extract respectively. Furthermore, uniform activity also been noticed on methanolic extract against SPMIC-29 and 130 Strains. From the present result showed that these are all the three experimental strains were significantly lowest susceptibility when compared with the STD drug of Gentamicin.

Table 2: *Zones of Inhibition Using Methanolic and Ethanolic Extracts of C. indicum (L.) Against Staphylococcus Strains*

Test (*Pseudomonas* strains)	Methanolic extract (Diameter mm)	Ethanolic extract (Diameter mm)	Ciprofloxacin 5mcg disc	Susceptibility to Ciprofloxacin	Negative control (Solvent only)
PA-37	6 ±1.54*	10 ±2.75**	3.1 ±0.52[Is]	S	-
PA-38	5±0.9*	10 ±2.65**	3 ±0.31*	S	-
A-39	9± 2.74*	8 ±1.56	0 ±0	R	-

**- Significant at 5% level*

***- Highly significant at 0.01% level*

R = Resistant, S = Sensitive, - = No zone of inhibition around the disc

[Is]- Insignificant

Similarly other experimental plant extract of *C. indicum* expressed maximum zone of inhibition against the SPMIC-130 (11±2.64) and SPMIC-132 (11±1.76) on both extracts. Though, in methanolic extract SPMIC-29 strains were expressed second most significantly highest activity. It was four fold greatest antibacterial responses compared with control disc of Gentamicin Table 2.

Table 3: *Bioactive spectrum of antibacterial activity in Methanolic and Ethanolic Extract of C. cinerariaefolium (L.) Against staphylococcus Strains*

Test (*Staphylococcus* strains)	Methanolic extract (Diameter mm)	Ethanolic extract (Diameter mm)	Gentamicin 10mcg disc	Susceptibility to the extract (Gentamicin)	Susceptibility to Ampicillin	Negative control (Solvent alone)
SPMIC-29	**10 ±0.45****	**9 ±1.54**	**6± 1.20**	**S**	**R**	**-**
SPMIC-130	**9 ±1.95**	**11 ±2.94***	**12± 3.65****	**S**	**R**	**-**
SPMIC-132	**11 ±1.76**	**6 ±0.84**	**9 ±1.65***	**S**	**R**	**-**

*- Significant at 5% level

**- Highly significant at 0.01% level

R = Resistant, S = Sensitive, - = No zone of inhibition around the disc

[Is]- Insignificant

When the *C. cinerariaefolium* ethanolic leaf extract treated with three different strains of *Pseudomonas* maximum similar zone of inhibition was noticed against PA-37 (10 ±2.75) and PA-38 (10 ±2.65). It was statistically significant as well as highest response when compared with Ciprofloxacin. Similarly, when the methanolic extract treated with this experimental strain PA-39 strain showed maximum effect followed by PA-37 (6 ±1.54) and 38 (5±0.9). From this table result showed interestingly accompanied with significantly increased worthy effect compared than Ciprofloxacin control or standard drug.

Table 4: *Bioactive spectrum of antibacterial activity of Zones around the Disc Using Methanolic and Ethanolic Extract of C. indicum (L.) flower against Pseudomonas Strains*

Test (*Pseudomonas* strains)	Methanolic extract (Diameter mm)	Ethanolic extract (Diameter mm)	Ciprofloxacin 5mcg disc	Susceptibility to Ciprofloxacin	Negative control (solvent alone)
PA-37	4 ±0.11*	10±1.36	13±0.36	S	-
PA-38	8 ±1.3**	9 ±0.65	13±2.14	S	-
PA-39	6±1.54*	11±2.68	0 ±0	R	-

*- Significant at 5% level

**- Highly significant at 0.01% level

R = Resistant, S = Sensitive, - = No zone of inhibition around the disc

Again the present study explained the second experimental organisms of three strains *Pseudomonas* strains showed that the maximum and minimum zone of inhibition was noticed on PA-38, 8±1.38 and PA- 37, 4±0.11 with similar methanolic solvent extract. Likewise ethanolic extract illustrated that the higher activity of this strain PA-39, 11±2.68 as well as PA-38, revealed the observed the value of 9±0.65 minimum effect on ethanolic extract. It was significantly lowest result when compared with standard disc Table 4.

Table 5: *Zones Around The Disc Using Methanolic And Ethanolic Extract of C. cinerariaefolium (L.) Against Candida Strains*

Test (*Staphylococcus* strains)	Methanolic extract (Diameter mm)	Ethanolic extract (Diameter mm)	Gentamicin 10mcg disc	Susceptibility to Gentamicin	Susceptibility to Ampicillin	Negative control (Solvent alone)
SPMIC-29	6 ±1.05	7 ±0.54	8 ±0.32	S	R	-
SPMIC-130	7 ±0.87*	3 ±0.41	2 ±1.22*	S	R	-
SPMIC-132	6± 1.65**	11 ±2.64**	10± 0.65**	S	R	-

Test (*Candida* strains)	Methanolic extract (Diameter mm)	Ethanolic extract (Diameter mm)	Susceptibility to Amphoterecin - B	Positive control (Aqueous Extract)
***C. tropicalis* (B-1389/09)**	9±0.65	12±1.36**	R	10±1.24
***C. albicans* (CAGMC6)**	8±1.10	10±1.01*	R	10±1.11
***C. albicans* (B-1622/09)**	7±1.65	7±2.30[Is]	R	9.31±0.98
***C. parapsilosis* (B1597/09)**	9±1.04*	4±0.64	R	11±1.76
***C. cruzei* (ATCC-6258)**	11±2.30**	8±1.60	R	8.64±1.02

*- Significant at 5% level

**- Highly significant at 0.01% level

R = Resistant, S = Sensitive, - = No zone of inhibition around the disc

[Is]- Insignificant

Table 6: *Zones around the Disc Using Methanolic Extract of C. indicum (L.) Leaves Against Candida Strain*

Test (*Candida* strains)	Methanolic extract (10µl) (Diameter mm)	Ethanolic extract (10µl) (Diameter mm)	Susceptibility to Amphoterecin- B	Positive control (Aqueous Extract)
***C. tropicalis* (B-1389/09)**	6±0.64*	15±1.65	R	10±0.52**
***C. albicans* (CAGMC6)**	4±1.54	4±1.32^{Is}	R	11±0.54**
***C. albicans* (B-1622/09)**	7±1.11	6±0.69	R	9±1.36*
***C. parapsilosis* (B1597/09)**	9±2.30	8±0.74*	R	10±1.65**
***C. cruzei* (ATCC-6258)**	4±1.41^{Is}	10±1.20**	R	11±2.31*

**- Significant at 5% level*

***- Highly significant at 0.01% level*

R = Resistant, S = Sensitive, - = No zone of inhibition around the disc

^{Is}- Insignificant

The Table 5 and Table 6 explained the result was antifungal activity against these two plant leaf extracts. When both the two plant with two solvent extracts treated in five various strains of the *Candida* species quietly highly significant zone of inhibition was observed against the strains such as *C. tropicalis*12±1.36 and *C. tropicalis*15±1.65 in Ethanolic extract from *C. cinerariaefolium* and *C. indicum* respectively. While the minimum antifungal activity was noticed on *C. parapsilosis* in ethanol extract from *C. Cinerariaefolium* also the similar lowest response observed on *C. albicans* (4±1.54) and *C. cruzei* (4±1.41) from the methanolic extract of *C. indicum* leaf.

Figure 1: *Chroamatogram view of C. indicum (L.) flower ethanolic extract by GCMS*

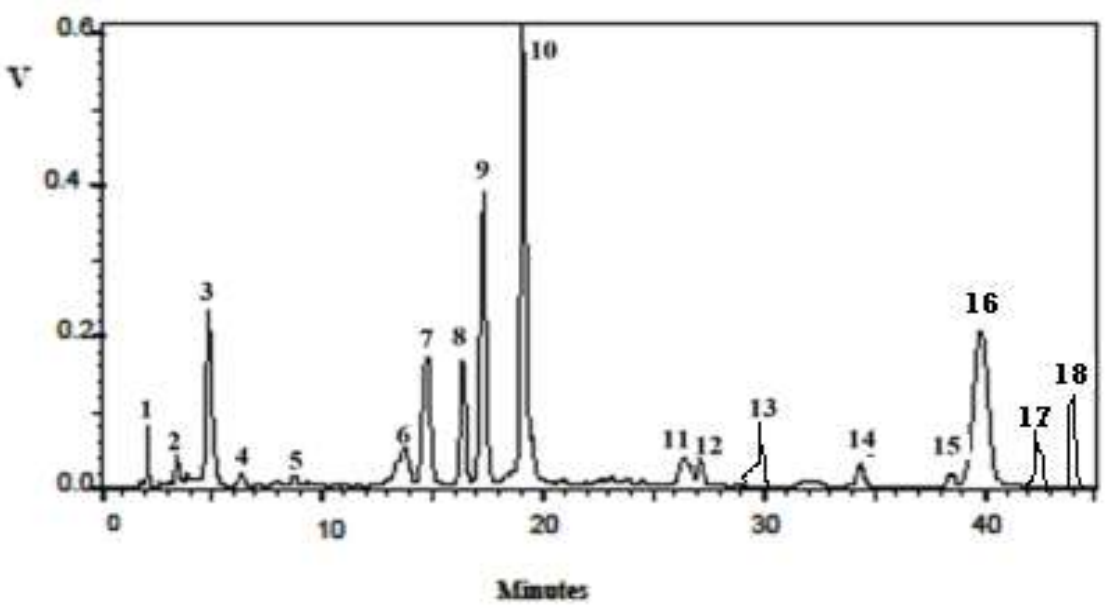

Table 7: *Compounds elucidation by GC – MS analysis of C. indicum (L.) flower ethanolic extract*

S.No.	Retention Time (RT)	Compound(s) separated	Abundance (%)
1	2.0	β- Elemene	0.1
2	3.5	1,8- Di cineole	0.03
3	5.0	γ- Cadinene Methyl ester	0.261
4	6.5	α- Copane	0.019
5	9.5	4- Terpineol ester	0.013
6	13.5	Sapthulenol	0.037
7	14.5	Diallyl 2- Isoborneol	0.180
8	16.5	5-ethyl-. Limonene	0.179
9	17.5	Isoborneol	0.410
10	19.5	camphor	0.60
11.	26.5	α- Gurjunene	0.021
12.	27.5	Sabinaketone	0.021
13.	29.5	-β- Farnesene	0.04
14.	34.5	Borneol	0.06
15.	38.5	β- Selinene	0.03
16.	40.5	Chrysanthenol	0.26
17.	42.5	α - Phellandrene	0.08
18.	44.5	Cis- β- Ocemene	0.14

Bioactive compound analysis by GC-MS and totally 18 bioactive compounds were identified. Amoung the 18 analytes camphor is an important peak compound also it contains 19.5 and 0.60% retention time and abundance respectively. Furthermore t *C. indicum* possessed secondly maximum compound is Isoborneol 0.410% of abundance and its retention time is 17.5, followed by other two compounds also been observed as a sub peak level such as γ- Cadinene Methyl ester and Chrysanthenol Figure 1. Eventhough, 4- Terpineol and β- Selinene compounds are very low amount present in the experimental flower (Table 7).

Many traditional healing herbs and plant parts have been shown to have medicinal value especially in the rural areas and these can be used to prevent and cure several human diseases. Even today, majority of the world population depends on herbal health care practice. Previously similar results also been depicted by [9] on other diverse organisms with different solvents such as the ethanolic extracts of *Chrysanthemum indicum* showed highest degree of inhibition against *Klebsiella pneumonia* and *Escherichia coli*, moderate degree of inhibition against *Streptococcus mutans, Psuedomonas aeruginosa, Bacillus subtilus* and minimum degree of inhibition against *Staphylococcus aureus*. The fungal culture of *Trichoderma viridae, Candida albicans, P. chrysogenum, Aspergillus niger* in petriplates were incubated along with the extract were checked for growth inhibitions zone of organisms. Similar kind of results also been published by [10][11].

It is interesting that was presence of Chrysanthenol, 5-ethyl- Limonene but the crude extracted still showed antibacterial activity [12]. Particularly *Chrysanthemum* family plant leaf and flowers prove the presence of promising antibacterial substances such as terpenoids, flavonoid, glysosides might be the good candidates for antibacterial activity against *B. cereus* and *L. monocytogenes* in both normal and osmotic stress (5% NaCl) even the antibacterial activity mechanism of andrographolide is still unclear and need to be further investigated this researched result was already conformed by [13][14][15].

CONCLUSION

From the present study undertaken the experimental, *Chrysanthemum* sp., such *as C. indicum, C. cinerifolium* flowers with two solvent extracts were studied for their bioefficacy on *Staphylococcal* species and *Pseudomonas* strain. The study revealed differences in their bioefficacy which might be attributed to the solvent used and also due to the presence of different types of secondary metabolites bioactive compounds present in the experimental flower was reported. Amoung the two plant flowers *C. indicum* possessed worthful antimicrobial activity than the *C. cinerifolium* flower. So from this study conclused that this *Chrysanthemum indicum* flower is act as a very good tool for the antimicrobial agent.

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