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Evaluation of antibacterial activity of three flower colours *Chrysanthemum morifolium* Ramat. against multi-drug resistant human pathogenic bacteria

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Key words: *Chrysanthemum morifolium*, Antibacterial activity, Pathogenic bacteria, MIC, MBC.

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Abstract

The present investigation was carried out to evaluate the anitibacterial activity of flower extracts of three colours (pink, yellow and white) of *Chrysanthemum morifolium* Ramat. against five Gram positive bacteria viz., *Staphylococcus aureus, Bacillus cereus, Streptococcus-β-haemolytica, Bacillus subtilis, Sarcina lutea* and five Gram negative bacteria viz., *Klebsiella* sp, *Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi, Shigella dysenteriae*. Between the two extracts (ethanol and methanol) the ethanol extracts of white flower was more effective than pink and yellow flower of *C. morifolium*. The ethanol extracts of white flower was showed the highest zone of inhibition (24.40 mm) against *Shigella dysenteriae*, the lowest MIC value (150 mg/ml) was against *Shigella dysenteriae* and *Streptococcus-β-haemolytica* and the lowest MBC value (200 mg/ml) was against *Shigella dysenteriae*. MIC and MBC of the extracts have ranged from 150-250 mg/ml and 200-300 mg/ml respectively. The lowest MIC and MBC values have observed against *Shigella dysenteriae*. For pink and yellow flower extracts, statistical results indicated that there are significant differences among bacterial species, solvent and bacterial strain, but no significant differences are shown in replication. But in case of yellow flower, there are significant differences among bacterial species, solvent, replication and bacterial strain. In addition, interaction between bacterial species and solvent appears to be significantly different.

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Introduction

Herbal medicines have received much attention as a source of new antibacterial drugs since they are considered as time-tested and comparatively state both for human use and the environment (Fazly-Bazzaz *et al*., 2005). There are typical phytochemicals found in chrysanthemum: volatile oil with such ingredients as borneol, camphor, chrysanthemum amino acids, etc. In addition, it also contains chrysanthemin, adenine, stachydrine, micro-vitamin A, vitamin B1, amino acids and acaclin, flavonoids, carotenoids, polyphenols etc. Some of the compounds in Chrysanthemum are flavonoids like luteolin, apigenin and acacetin, choline, and vitamin B_1. It is also a good source of Vitamins C and A, Niacin, Folic acid and Pantothenic acid and is also rich in calcium, magnesium, potassium, iron and phosphorus. Chrysanthemum tea can help detoxify blood, regulate blood pressure and calm the nerves. It has antibacterial properties that can be effective against *Staphylococcus aureus, Streptococcus hemolyticus B, Dermatomycosis, Shigella dysenteriae* and *Tubercle bacillus*. *C. morifolium* was found to have high amounts of chlorogenic acid, flavonoids glucosides (including acetyl glucoside, neohesperiidoside) and apigenin. Several research shows that chrysanthemum contain significant antibacterial activity. *C. morifolium* flowers afforded mixtures of the C-3 palmitate and myristate esters (3:2) of heliantriol C (2) and fatty acid esters (1:1) of faradiol (3) and arnidol (Ragasa *et al*., 2005). Toppo *et al*. (2015) investigated that this plants used in traditional medicine may constitute an important source of new biologically active compounds. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanism of action for new and re-emerging infectious diseases. In their study they carried out the antimicrobial effect and analysis of phytochemical constituents of different plant parts of *Chrysanthemum morifolium* Ramat. Pathogenically significant bacteria (*Salmonella typhi*-MTCC-733, *Staphylococcus aureus*- MTCC-7443, *Pseudomonas aeruginosa*-MTCC-7296, *Mycobacterium tuberculosis*-MTCC-300) and fungus (*Microsporum canis*-MTCC-2820, *Epidermophyton floccossum*-MTCC-613, *Trichophyton rubrum* -MTCC-296 and *Aspergillus candidus* -MTCC-1989) were selected for their study. There are no evidence of finding and comparing atnibacterial properties of three flower colours (pink, yellow and white) of *C. morifolium*. From this point of view, the present study was undertaken to determine the antibacterial activity of flower extracts of three colours (pink, yellow and white) *C. morifolium against multi drug resistant human pathogenic bacteria*.

Materials and methods

Plant collection and identification

White and yellow colour flower of *Chrysanthemum morifolium* were collected from local farmer of Jessore, Bangladesh and pink colour flower of *chrysanthemum morifolium* was collected from local farmer of Rajshahi, Bangladesh. The plant material was collected in December-January, 2014. Dr. A. H. M. Mahbubur Rahman, Associate Professor, Department of botany, University of Rajshahi-6205, Bangladesh, confirmed the taxonomic identification of the plant.

Preparation of powder

Collected three colour (pink, yellow and white) of flowers were washed with clean sterile distilled water, and dried for 3 days in oven under 60°C to reduce water content. Then the dried plant materials were crushed into fine powder using mortar, pestle and electric blender (Nokia, Osaka-Japan).

Preparation of flower extracts

The powdered plant materials were extracted with methanol and ethanol. Fifty gram fine powder was dipped into 150 ml methanol and 150 ml ethanol into different conical flask stoppered with rubber corks and left for full 3 days with constant shaking using orbital shaker. After 3 days, the resulting mixtures were than filtered into two stages. First, Teton cloth was used and secondly Whatman No. 1 filter paper was used for more delicate filtration. Filtrates were taken into glass beaker for evaporating solvent (methanol and ethanol).

For quick evaporation of extra solvent from the extracts, Water bath (4 holes analogue, thermostatic water bath, China) was used under 60 °C. Semi solid filtrates were dissolved in respective solvent and transferred into airtight screw cap tube and stored at 4 °C (Akueshi *et al*., 2002). To calculate yield (final semi solid material) performance of the extract, standard formula was used according to Ekwenye and Elegalam (2005).

Formula: $\frac{\text{Yield} \times 100}{\text{Fine powder weight}} = \text{Yield (\%)}$

Bacterial strains

Five Gram positive bacteria namely, *Staphylococcus aureus* **(**BMLRU1002), *Bacillus cereus* (BMLRU1004), *Streptococcus-β- haemolytica* (BMLRU1006), *Bacillus subtilis* (BMLRU1008), *Sarcina lutea* (BMLRU1012) and five Gram negative bacteria namely, *Klebsiella* sp. (BMLRU1003), *Klebsiella pneumonia* (BMLRU1005), *Pseudomonas aeruginosa* (BMLRU1007), *Salmonella typhi* (BMLRU1009), *Shigella dysenteriae* (BMLRU1011) were used for antibacterial study. All of the tested bacterial species were collected from the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B), Mohakhali, Dhaka 1212, Bangladesh.

Antibacterial assay

Antibacterial effect of the selected flower extracts were attempted by the disc diffusion assay (Kirby-Bauer Method) was used to screen antibacterial activity (Bauer *et al*., 1966; Barry, 1980). Sterilized filter paper discs (6 mm in diameter) were soaked with 10 µl of methanol and ethanol extracts and dried under aseptic condition inside the laminar flow. 30 µl of standard bacterial cultures (approximately 10^8 cfu/ml; 0.5 McFarland turbidity standards) were spread on agar plates. Negative controls were prepared using the respective solvents. Ciprofloxacin (30 µg $disc^{-1}$) was used as positive control. After drying in air under aseptic condition discs were placed on seeded agar plates and incubated at 37 °C for 24 h. Antibacterial activity was evaluated by measuring the diameter of zones of inhibition (mm) against the tested bacteria. Each assay was carried out in triplicates.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) of the extracts were determined according to (Doughari *et al.,* 2007). The Minimum Inhibitory Concentration (MIC) was determined for each of the test organisms in triplicate in test tubes. To 0.5 ml of varying concentrations of the extracts (100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600 mg/ml) in test tubes, nutrient broth (2 ml) was added and then a loop-full of the test organism, previously diluted to 0.5 McFarland turbidity standards, was introduced. The procedure was repeated on the test organisms using the standard antibiotics (ciprofloxacin). A tube containing nutrient broth only was seeded with the test organisms, as described above, to serve as controls. The culture tubes were then incubated at 37 °C for 24 h. After incubation the tubes were then examined for microbial growth by observing for turbidity. To determine the MBC, for each set of test tubes in the MIC determination, a loop-full of broth was collected from those tubes that did not show any growth and inoculated onto sterile nutrient agar by streaking. Nutrient agar plates only were also streaked with the respective test organisms to serve as controls. All the plates were then incubated at 37 °C for 24 h. After incubation the concentration at which no visible growth was seen was noted as the Minimum Bactericidal Concentration (MBC).

Statistical analysis

Antibacterial activity of flower extracts were statistically analyzed using analysis of variance (ANOVA). All the results are represented as means ± SE of three independent replications. Calculated F-values were compared with critical F-value/Table value in 5% significant level.

Results

The antibacterial activity of three colour of flower extracts of *C. morifolium* against ten human pathogenic bacteria is shown in Table 1; Table 2 and Table 3 respectively.

The studied concentrations of extract exhibited different degrees of antibacterial activity depend on bacterial strains and solvents compared with the reference standard antibiotic and more activity observed with ethanol extracts.

Negative control (disc containing only solvent methanol) exhibits no zone of inhibition against the entire tested organisms. But positive control exhibits zone of inhibition against all the tested organisms and the range of zone was 26.66 mm - 31.66 mm.

Table 1. Antibacterial activities of pink flower extracts of *C. morifolium* against ten Human pathogenic bacteria.

Bacterial species		Zone of inhibition (mm)			
		Methanol extracts (800 mg/ml)	Ethanol extracts (800 mg/ml)	Positive control (30 µg/ml)	Negative control
Gram Positive	*Staphylococcus aureus*	13.30±0.67	16.33±0.57	30.33±0.33	-
	Bacillus cereus	17.10±0.88	14.90±0.57	29.66±0.51	-
	Streptococcus-β-haemolytica	11.50±0.88	14.80±0.50	30.33±0.51	-
	Bacillus subtilis	12.33±0.57	16.00±0.50	29.33±0.19	-
	Sarcina lutea	16.44±0.88	17.50±0.67	31.00±0.16	-
Gram Negative	*Klebsiella* sp.	14.00±0.50	16.66±0.57	30.33±0.33	-
	Klebsiella pneumonia	15.92±0.88	20.00±0.88	31.00±0.29	-
	Pseudomonas aeruginosa	13.83±0.33	22.33±0.66	30.66±0.25	-
	Salmonella typhi	13.35±0.58	24.33±0.57	31.33±0.33	-
	Shigella dysenteriae	15.92±0.88	18.50±0.50	29.66±0.33	-

Note: Data are represented as mean ± SE of triplicate experiments; (-) = No inhibition.

Table 1 Continued. Statistical analysis (ANOVA).

Source of variation	df	SS	MS	F	Comment
Bacterial species	9	122.008	13.556	57.178	***
Solvant	2	4219.153	2109.576	8897.747	***
Bacterial strain	1	58.936	58.936	248.578	***
Replication	2	1.096	0.548	2.310	Ns
Bacterial species X Solvent	18	209.958	11.664	49.198	***
Error	57	13.514	0.237		
Total	89	4624.664			

Note: * =significant; Ns = not-significant.

For pink flower, ethanol pink flower extract showed highest zone of inhibition (24.33mm) against gram negative bacteria *Salmonella typhi* and methanol extract showed the highest zone of inhibition (17.10mm) against gram positive bacteria *Bacillus cereus* (Table 1).

Here, ethanol extract of Yellow flower of chrysanthemum showed the highest zone of inhibition (23.33mm) against *Klebsiella pneumoniae* and in case of methanol extract also showed the highest zone of inhibition (13.70mm) against the same bacteria (Table 2).

Table 2. Antibacterial activities of yellow flower extracts of *C. morifolium* against ten Human pathogenic bacteria.

Bacterial species		Zone of inhibition (mm)			
		Methanol extracts (800 mg/ml)	Ethanol extracts (800 mg/ml)	Positive control (30 µg/ml)	Negative control
Gram Positive	*Staphylococcus aureus*	10.33±0.63	15.70±0.60	29.67±0.33	-
	Bacillus cereus	10.86±0.80	16.66±0.57	31.66±0.35	-
	Streptococcus-β-haemolytica	10.33±0.57	18.50±0.50	30.33±0.33	-
	Bacillus subtilis	11.33±0.57	14.50±0.50	30.66±0.33	-
	Sarcina lutea	10.60±0.60	22.33±0.57	29.33±0.29	-
Gram Negative	*Klebsiella* sp.	11.60±0.60	16.33±0.57	29.00±0.33	-
	Klebsiella pneumonia	13.70±0.60	23.33±0.57	31.33±0.33	-
	Pseudomonas aeruginosa	12.50±0.50	15.00±0.64	30.33±0.33	-
	Salmonella typhi	12.50±0.50	15.67±0.57	29.33±0.33	
	Shigella dysenteriae	11.30±0.26	22.66±0.57	31.33±0.33	-

Note: Data are represented as mean ± SE of triplicate experiments; (-) = No inhibition.

Table 2 Continued. Statistical analysis (ANOVA) of

Source of variation	df	SS	MS	F	Comment
Bacterial species	9	136.920	15.213	46.658	*
Solvant	2	5441.452	2720.726	8344.203	*
Bacterial strain	1	13.348	13.348	40.937	*
Replication	2	2.539	1.269	3.893	*
Bacterial species X Solvent	18	221.652	12.314	37.766	*
Error	57	18.586	0.326		
Total	89	5834.495			

Note: * =significant; Ns = not-significant.

Statistical result indicated that there are significant differences among bacterial species, solvent, replication and bacterial strain. In addition, interaction between bacterial species and solvent shown that they were significantly different (Table 2).

On the other hand, considering the two extract of White flower, ethanol extract showed highest zone of inhibition (24.40mm) against gram negative bacteria *Shigella dysenteriae* and for methanol extract the highest zone of inhibition (14.50 mm) was against gram negative bacteria *Salmonella typhi (*Table 3).

Table 3. Antibacterial activities of white flower extracts of *C. morifolium* against ten Human pathogenic bacteria.

Bacterial species		Zone of inhibition (mm)			
		Methanol extracts (800 mg/ml)	Ethanol extracts (800 mg/ml)	Positive control (30 µg/ml)	Negative control
Gram Positive	*Staphylococcus aureus*	11.50±0.88	18.50±0.50	28.33±0.33	-
	Bacillus cereus	10.33±0.50	16.33±0.57	29.60±0.35	-
	Streptococcus-β-haemolytica	14.00±0.58	22.33±0.57	26.66±0.33	-
	Bacillus subtilis	12.50±0.50	14.33±0.57	31.60±0.23	-
	Sarcina lutea	11.30±0.26	13.50±0.50	30.33±0.33	-
Gram Negative	*Klebsiella* sp.	12.66±0.57	15.66±0.57	30.50±0.29	-
	Klebsiella pneumonia	12.67±0.57	15.50±0.50	30.50±0.29	-
	Pseudomonas aeruginosa	10.66±0.57	17.50±0.50	30.40±0.60	-
	Salmonella typhi	14.50±0.50	21.33±0.57	30.33±0.29	-
	Shigella dysenteriae	13.30±0.67	24.40±0.60	29.60±0.35	-

Note: Data are represented as mean ± SE of triplicate experiments; (-) = No inhibition.

Table 3 Continued. Statistical analysis (ANOVA).

Source of variation	df	SS	MS	F	Comment
Bacterial species	9	185.672	20.630	41.847	*
Solvant	2	5034.327	2517.163	5105.853	*
Bacterial strain	1	17.885	17.885	36.277	*
Replication	2	1.629	0.815	1.652	Ns
Bacterial species X Solvent	18	216.000	12.000	24.341	*
Error	57	28.101	0.493		
Total	89	5483.613			

Note: * =significant; Ns = not-significant.

Comparative study among three colours flower of chrysanthemum in two solvents against tested bacteria at highest concentration (800 mg/ml)

Results of comparative study among three colours (white, pink and yellow) flower of chrysanthemum in two solvents (methanol and ethanol) against tested bacteria at highest concentration (800 mg/ml) are shown in Figure 1 and 2.

The result showed that, white, pink and yellow flower extract of ethanol and methanol against ten human pathogenic bacteria. In case of gram positive bacteria *Staphylococcus aureus*, ethanol white flower extracts are more effective than others. For *Bacillus cereus*, ethanol yellow flower extracts are more effective than others. Furthermore, ethanol white flower is more effective against *Streptococcus-β- haemolytica* than others.

Moreover, ethanol pink flower is more effective against *Bacillus subtilis* bacteria than other colours flower in methanol and ethanol solvents, and ethanol yellow flower is more effective against *Sarcina lutea* bacteria than others.

Table 4. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of pink flower extracts of *C. morifolium*.

	Bacterial strain	Methanol extract		Ethanol extract	
		MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Gram positive	*Staphylococcus aureus*	250	300	200	250
	Bacillus cereus	150	200	250	250
	Streptococcus-β-haemolytica	250	300	200	250
	Bacillus subtilis	250	300	150	200
	Sarcina lutea	150	250	150	250
Gram negative	*Klebsiella* sp.	250	300	250	300
	Klebsiella pneumonia	200	250	200	250
	Pseudomonas aeruginosa	250	300	150	250
	Salmonella typhi	250	300	150	200
	Shigella dysenteriae	200	250	200	250

On the other hand, in case of gram negative bacteria, *klebsiella sp*, among these three flower colours of methanol and ethanol extracts, ethanol pink flower extracts are more effective than others. In case of *Klebsiella pneumoniae,* among these three flower colours of methanol and ethanol extracts, ethanol yellow flower extracts are more effective than others. Furthermore, ethanol yellow flower is more effective against *Pseudomonas aeruginosa*. Moreover, ethanol pink flower is more effective against *Salmonella typhi* bacteria than other colours flower in methanol and ethanol solvents, and ethanol white flower is more effective against *Shigella dysenteriae* bacteria.

Table 5. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of yellow flower extracts of *C. morifolium*.

	Bacterial strain	Methanol extract		Ethanol extract	
		MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Gram positive	*Staphylococcus aureus*	250	300	250	300
	Bacillus cereus	250	300	250	300
	Streptococcus-β-haemolytica	250	250	200	250
	Bacillus subtilis	250	300	250	300
	Sarcina lutea	250	300	150	200
Gram negative	*Klebsiella* sp.	250	300	200	250
	Klebsiella pneumonia	150	200	150	200
	Pseudomonas aeruginosa	200	250	250	300
	Salmonella typhi	150	250	250	300
	Shigella dysenteriae	250	300	150	200

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The result showed that in pink flower extracts of *C. morifolium* (Table 4), the range of MIC values of methanol extracts were 150-250 mg/ml against test of the bacterial strains, and range of MBC values were 200-300 mg/ml. The range of MIC values of ethanol extracts were 150-250 mg/ml, and range of MBC values were 200-300 mg/ml. The ethanol extract of Pink flower showed the lowest MIC values 150 mg/ml against *Bacillus subtilis*, *Sarcina lutea, Pseudomonas aeruginosa* and *Salmonella typhi*, and lowest MBC values 200 mg/ml

showed against *Bacillus subtilis* and *Salmonella typhi*. In contrast, methanol extract of Pink flower showed the lowest MIC values against *Bacillus cereus, Sarcina lutea* and lowest MBC value showed against *Bacillus cereus*.

Table 6. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of white flower extracts of *C. morifolium*.

Bacterial strain		Methanol extract		Ethanol extract	
		MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Gram positive	*Staphylococcus aureus*	250	300	200	250
	Bacillus cereus	250	300	250	300
	Streptococcus-β-haemolytica	250	250	150	250
	Bacillus subtilis	250	300	250	300
	Sarcina lutea	250	300	250	350
Gram negative	*Klebsiella* sp.	250	300	250	300
	Klebsiella pneumonia	150	200	250	300
	Pseudomonas aeruginosa	200	250	200	250
	Salmonella typhi	150	250	200	250
	Shigella dysenteriae	250	300	150	200

On the other hand, in yellow flower extracts of *C. morifolium*, the result showed that (Table 5), the range of MIC values of methanol extracts were 150-250 mg/ml against test of the bacterial strains, and range of MBC values were 200-300 mg/ml. The range of MIC values of ethanol extracts were 150-250 mg/ml, and range of MBC values were 200-300 mg/ml. Ethanol extract of Yellow flower showed the lowest MIC (150 mg/ml) and MBC (200 mg/ml) values against *Sarcina lutea, Klebsiella pneumoniae, Shigella dysenteriae*. In contrast, methanol extract of Yellow flower of chrysanthemum showed the lowest MIC values against *Klebsiella pneumoniae, shigella dysenteriae* and lowest MBC values for *Klebsiella pneumoniae*.

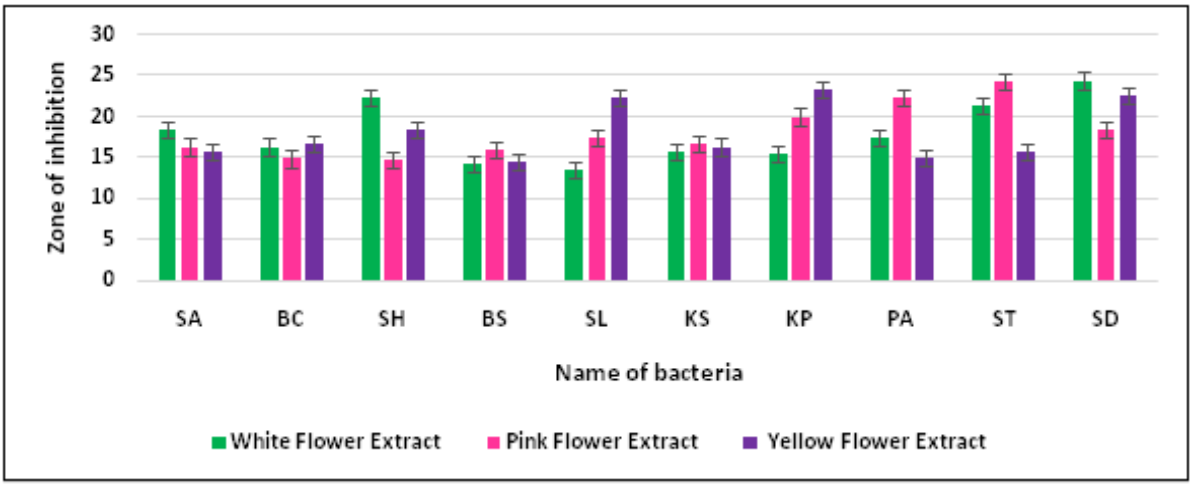

Fig. 1. Comparison of zone of inhibition among ethanol white, pink and yellow flower extracts of *C. morifolium* against tested bacteria at highest concentration (800 mg/ml).
(SA= *Staphylococcus aureus,* BC= *Bacillus cereus,* SH= *Streptococcus-β-haemolytica,* BS= *Bacillus subtilis,* SL= *Sarcina lutea,* KS= *Klebsiella* sp., KP= *Klebsiella pneumonia,* PA= *Pseudomonas aeruginosa,* ST= *Salmonella typhi,* SD= *Shigella dysenteriae*.).

Moreover, in white flower extracts of *C. morifolium*, the result showed that (Table 6), the range of MIC values of methanol extracts were 150-250 mg/ml against test of the bacterial strains, and range of MBC values were 250-300 mg/ml. The range of MIC values of ethanol extracts were 150-250 mg/ml, and range of MBC values were 200-300 mg/ml. The ethanol extract of White flower of chrysanthemum showed the lowest MIC values 150 mg/ml against *Streptococcus-β-haemolytica* and *Shigella dysenteriae* and lowest MBC value 200 mg/ml showed against *Shigella dysenteriae*. In contrast, lowest MIC (200 mg/ml) and MBC (250 mg/ml) values were found in methanol extract of White flower against *Streptococcus-β-haemolytica, Klebsiella pneumoniae, Salmonella typhi and Shigella dysenteriae*.

Discussion

The present study was conducted to obtain preliminary information on actibacterial activity of three different colours (pink, yellow and white) flowers of *Chrysanthemum morifolium* have been determined antibacterial activities against the sensitivity of ten pathogenic bacteria (five gram positive bacteria and five gram negative bacteria) and compared to antibiotic Ciprofloxacin.

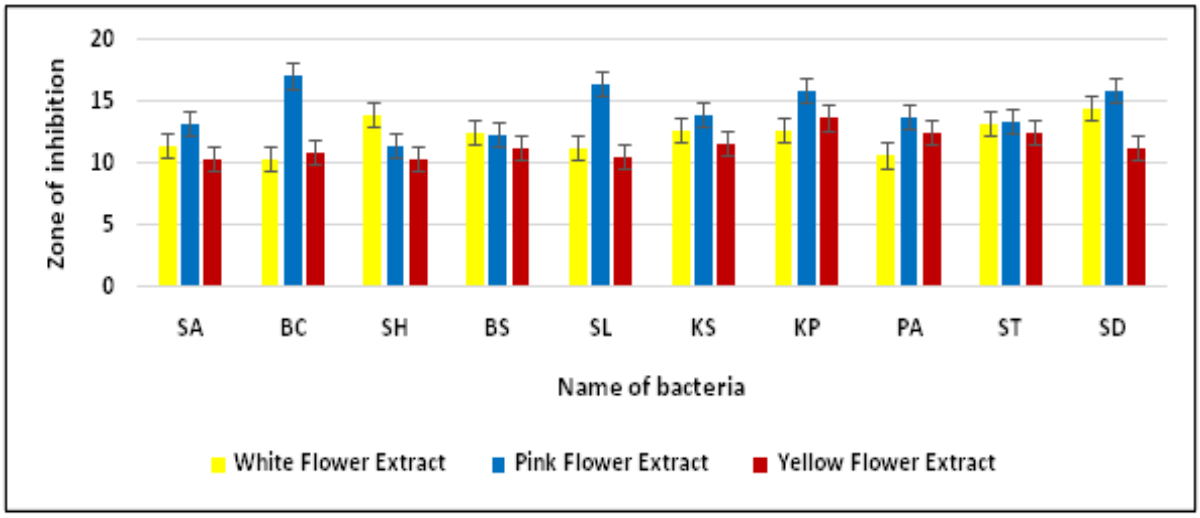

Fig. 2. Comparison of zone of inhibition among methanol white, pink and yellow flower extracts of *C. morifolium* against tested bacteria at highest concentration (800 mg/ml)
(SA= *Staphylococcus aureus,* BC= *Bacillus cereus,* SH= *Streptococcus-β-haemolytica,* BS= *Bacillus subtilis,* SL= *Sarcina lutea,* KS= *Klebsiella* sp., KP= *Klebsiella pneumonia,* PA= *Pseudomonas aeruginosa,* ST= *Salmonella typhi,* SD= *Shigella dysenteriae*.

The inhibition zones were varied at different concentrations. The widest inhibition zone of ethanolic extract of pink flower was 24.33 mm against *Salmonella typhi* .While the widest zone of methanolic extract of pink flower was 17.10 mm against *Bacillus cereus*. On the other hand, the widest zone of ethanolic extract of yellow flower was 23.33 mm against *Klebsiella pneumonia*. While the widest zone of methanolic extract of yellow flower was 13.70 mm against *Klebsiella pneumonia* and the widest inhibition zone of ethanolic extract of white flower was 24.40 mm against *Shigella dysenteriae*.

While the widest zone of methanolic extract of white flower was 14.50 mm against *Salmonella typhi* .The results pointed out that the ethanolic extract showed higher zone of inhibition followed by methanol. This plants with anti-bacterial effects are rich in polyphenolic substances such as tannins, catechins, alkaloids, steriods and polyphenolic acids. The anti-bacterial activity also could be due to various chemical components and the presence of essential oils in adequate concentrations, which damage micro-organisms (Deininger *et al*., 1984). The essential oils obtained by hydrodistillation from the flowers and leaves of *Chrysanthemum morifolium* Ramat.

were analyzed by gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC/MS) (Oladipupo *et al.*, 2014). The essential oils were evaluated against both Gram positive (*Enterobacter cloacae* ATCC 13047, MRSA (Methicillin-resistant *Staphylococcus aureus*), *Staphylococcus aureus* ATCC 25923) and six Gram negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas syringae, Salmonella sp, Serratia liquefaciens* ATCC 27592, *Serratia marcescens* ATCC 14756, *Shigella sp*). The extract contains many phytochemicals substances including terpenoids, tannins and polyphenolic compounds as well as flavonoids which have a potential antimicrobial activity. Flavonoid's activity is probably due to their ability to complex with extra cellular and soluble proteins and to complex with bacterial cell walls and lipophilic flavonoids may also disrupt bacterial membranes. Gram negative bacteria have been found to be less susceptible to plant extracts in earlier studies done by other researchers (Kuhnt *et al.*, 1994; Afolayan and Meyer 1995). In this study, Gram negative bacteria is more prominent than Gram positive bacteria and extracts showed different degrees of growth inhibition depending upon the bacterial strains. These variations were found because strains are genetically different from each other, and this is probably due to the differences in chemical composition and structure of the cell wall of both types of microorganisms (Kaushik and Goyel, 2008). This may be attributed to the fact that these two groups differ by its cell wall component and its thickness (Yao *et al.* 1995). Increasing of the concentrations level of extracts had a significant ($P<0.05$) inhibitory effect on all studied bacteria. This observation is very significant because of the possibility of developing therapeutic substances that will be active against multidrug-resistant organisms. It is also noticeable that ethanol extract is more effective than methanol. This may be due to the better solubility of the active components in the solvents (De Boer *et al.*, 2005). These include differences in microbial growth, exposure of microorganisms to plant extracts, the solubility of extracts or extracts components and the use and quantity of an emulsifier (Bansod and Rai 2008).

However, the difference between the present study and others done by various scientist might be due to differences in the methodology or the difference in the solvent used for extraction of the sample. The results suggest that pink, white and yellow flower of chrysanthemum contain active ingredients which qualify them for medicinal use.

Conclusion

In the present study, flower extracts of (methanol and ethanol) pink, yellow and white flower of chrysanthemum presented a significant percentage zone of inhibition against ten pathogenic bacteria. The overall results showed that ethanol extracts of white was more effective for bacteria than others. The maximum zone of inhibition was observed in ethanol extracts of white (24.40 mm) against *Shigella dysenteriae* with the lowest MIC value (150 mg/ml). It can be concluded that this three colour of flowers investigated, have opened up a new perspective in pharmaceutical research and they can be used for the development of potential, novel antimicrobial agents for the treatment of microbial diseases. They are novel source of medicines as they have a reservoir of chemical agents with therapeutic properties (Sandigawad 2010) and plants are the cheapest and safer alternative sources of antimicrobials (Kumar *et al.*, 2012) Plant extracts have both phytochemical and antimicrobial properties and can be of great significance in therapeutic treatments (Nagesh *et al.*, 2009).

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