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Phytochemical analysis and antioxidant activity of three flower colours *Chrysanthemum morifolium* Ramat.

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Abstract

Chrysanthemum morifolium Ramat. is an important medicinal plant. To learn more about properties of this plant, the present study was aimed to evaluate the phytochemical analysis and antioxidant activity of three colours (pink, yellow and white) flower of *Chrysanthemum morifolium*. Spot phytochemical screening of these three colours of *C. morifolium* in the ethanol extract showed that alkaloids, phenols, flavonoids, glycoside and terpenoids were present in studied three colours (pink, white and yellow) flower but saponin and tannin were absent in pink and yellow flower. Free radical-induced oxidative stress is the root cause for many human disease. Naturally occurring antioxidant supplements from plants are vital to counter the oxidative damage in cells. An antioxidant activity of these three flower colours (pink, yellow and white) *Chrysanthemum morifolium* were examined through DPPH antioxidant assay. The sample showed significant antioxidant activity. Where IC_{50} value of the three flower extracts were 10.00mg/ml, 11.00mg/ml and 40.00mg/ml for white, pink and yellow flower extracts respectively. In ascorbic acid the IC_{50} value was calculated 11.50 mg/ml. The phytochemical analysis supports antioxidant properties of these flower colours. So, for comparing among pink, white, yellow flower, white flower of chrysanthemum was found good for potential phytochemical and antioxidant properties than pink and yellow flower.

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Introduction

Chrysanthemum morifolium Ramat. is a perennial plant belonging to the asteraceae family. It has been cultivated for more than 3000 years. Chrysanthemum has antioxidant, anti-inflammatory, antimutagenic, antimicrobial, antifungal, antiangiogenic, antiatheroslerosis and nematicidal properties. 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. Chrysanthemum and its herbal infusions are used in the treatment of bacterial and viral infections, sinusitis, blood pressure, digestive, skin problems, influenza virus PR3, leptospira, HIV-1, human colon cancer Colon205 cells, headache, dizziness, sore throat, hypertension, flu, cough etc.

Phytochemicals are bioactive compounds found in plants that work with nutrients and dietary fiber to protect against diseases. They are non-nutritive compounds. These phytochemicals are the secondary metabolities present in smaller quantities in higher plants and they include the alkaloids, steroids, flavonoids, terpenoids, tannins and many others (Peteros, 2010). Phytonutrients have various health benefits, for example, they may have antimicrobial, anti-inflammatory, cancer preventive, antidiabetic and antihypertensive effects to mention but a few. It is crucial to know the type of phytochemical constituent, thus knowing the type of biological activity which might be exhibited by the plant (Agbafor and Nwachukwu, 2011). The importance of medicinal plants and the contribution of phytomedicine to the well-being of a significant member of the world's population have attracted interest from diverse disciplines. Phytochemical test and comparing among three different colours flower of chrysanthemum was not worked in before that we worked.

Medicinal plants produce a vast array of secondary metabolites (Croteau *et al.*, 2000) and such metabolites have been found to have a broad range of therapeutic properties (Cragg *et al.*, 2005), including antioxidant activities (Chin *et al.*, 2009). Through oxidation reactions, living cell generates a number of reactive oxygen species (ROS) like superoxide, hydroxyl, peroxyl, alkoxy, nitric oxide etc., which induce oxidative stress and initiate chain reactions leading to cell damage and various diseases (Fang *et al.*, 2002) while antioxidants prevent the oxidation of other molecules, cancel out the cell-damaging effects of free radicals (Sies *et al.*, 1997) and lower the risk of different diseases. Several enzymes as well as non-enzymatic secondary metabolic compounds of plant origin are able to scavenge ROS (Blokhina *et al.*, 2003) and thus can protect the organism from oxidative damage.

Free radicals are a major interest for physiological and biochemical lesions. Antioxidants inhibit or prevent oxidation of substrates and evolve to protect biological systems against damage induced by ROS. Interest in finding naturally occurring antioxidants in foods or medicines to replace synthetic antioxidants has increased considerably, given that synthetic antioxidants are being restricted due to their side effects (Zheng and Wang, 2001). Therefore, interest in finding natural antioxidants, without undesirable side effects, has increased greatly. The numbers of antioxidant compound by plants play important roles in preventing diseases induced by free radicals (Hirose *et al.*, 1994). Therefore, attention has been directed toward the development of natural antioxidants from plant sources (Chou *et al.*, 2009; Lin *et al.*, 2010) In the present work, ethanol extracts of three colours (pink, yellow and white) flower of *Chrysanthemum morifolium* were examined for their secondary metabolites content and antioxidant activities.

Materials and methods

Plant material

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, Rajshahi University Rajshahi-6205, Bangladesh, confirmed the taxonomic identification of the plant.

Chemicals and Reagents

2, 2-Diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, methanol, ethanol, Dragendroff's, Hager's, Mayer's, Wagner's and Tannic acid reagents.

Spot phytochemical analysis

Preparation of sample

Initially well washed flowers were dried in hot air oven. The dried materials were coarsely powdered as a fine powder. Spot phytochemical screening of three colours (pink, yellow and white) flower of *C. morifolium* were carried out by using the following protocols as described below for the presence of alkaloids, flavonoids, glycosides, saponins, tannins, terpenoids and phenols.

(a) Determination of Alkaloids

5 g fine powder was mixed up to moistered with 10 ml 2% HCl and heated in water at 60 ˚C for one hour. After cooling the sample was filtered through Whatmann No. 1 filter paper. Two drops of filtrates were put on a microscopic groove slide with one drop of the alkaloid detecting reagent. The relative abundance of precipitate, if any, formed in the plant sample with the reagents was considered as the presence of alkaloid (Aplin and Cannon 1971).

(b) Determination of Flavonoids

About 10 g plant sample was extracted with 20 ml ethanol (1:2) in aspirator bottle by soaking about 72 hours. After that few drops of conc. HCl were added to the alcoholic extract resulting red color, indicates the presence of flavonoids (Farnsworth 1985).

(c) Determination of Glycosides

A small amount of sample solution was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2ml of concentrated H_2SO_4. A brown ring at the interphase indicated the presence of cardiac glycosides (Trease and Evans 1989).

(d) Determination of Saponins

20 ml water is added to 150 mg fine powder and shaken vigorously; layer of foam formation indicates the presence of saponins (Siddiqui and Ali 1997).

(e) Determination of Tannins

2g fine powder was extracted with 10 ml distilled water (1:5), and was boiled for about 20 to 25 minutes. After cooling the extract was filtered. The filtrate was taken on 3 microscopic slides, two drops on each. Then to the first slide one drop 10% NaCl, to the second 1% gelatin and to the third 1% gelatin + 10% NaCl were added. The appearance of a white precipitate on the second and third was taken as positive test for tannins (Wall *et al.*1954).

(f) Determination of Terpenoids

2ml of sample was mixed in 5ml of chloroform and concentrated $H2SO_4$ 2ml was carefully added to form layer. A reddish brown coloration of the interface was formed show positive result for the presence of terpenoids (Harborne 1973).

(g) Determination of Phenols

5ml of sample was mixed with 2ml of 2% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols.

Antioxidant activity

Antioxidant activity of three colours (pink, yellow and white) flower of *C. morifolium* were estimated for their free radical scavenging activity by using DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) as described by Hsu *et al.* (2007) with some modification.

Experimental procedure of Antioxidant activity test

First, various concentrations like 20, 40, 60, 80, 100 mg/ml of sample in methanol were prepared. 2 ml of methanol solution of plant sample or standard at different concentrations was taken in test tube. 3 ml of 0.1 mm methanol solution of DPPH was added into the test tube.

The test tube was incubating at room temperature for 30 minutes in dark place to complete the reaction. Then the absorbance of the solution was measured at 517 nm using a spectrophotometer against control. Ascorbic acid was used as standards (positive control). Then the percentage (%) inhibition activity was calculated according to Pavlov *et al.* (2002).

$$\% I = \{(A_0 - A_1)/A_0\}^*100$$

Where, A_0 is the absorbance of the control, and A_1 is the absorbance of the sample or standard. Sample was analyzed in two replications and data presented as mean (±) standard deviation (SD). Then % of inhibition was plotted against blank concentration and from the graph IC_{50} was calculated. IC_{50} value, the concentration of sample required for 50% scavenging of DPPH free radical are completed (Mandal *et al.*, 2009).

Statistical analysis

Statistical analysis (ANOVA) and Least Significant Difference (LSD) test was used to speculate further if there was a significant difference within varieties, various concentrations. P values <0.05 were considered as significant.

Results

Spot phytochemical analysis

Spot phytochemical analysis of three colours (pink, yellow and white) flower of *Chrysanthemum morifolium* were examined qualitatively for their alkaloid, flavonoid, glycoside, terpenoid, saponin, tannin and phenol content.

The result has revealed the presence of alkaloids, flavonoids, glycoside, terpenoids and phenols were present in studied three colours (pink, white and yellow) flower but saponin and tannin were absent in pink and yellow flower (Table 1).

Table 1. Spot phytochemical screening of three colours (pink, yellow and white) flower of *C. morifolium*.

Phytochemicals	White flower	Pink flower	Yellow flower
Alkaloids	+	+	+
Flavonoids	+	+	+
Glycosides	+	+	+
Saponins	+	-	-
Tannins	+	-	-
Terpenoids	+	+	+
Phenols	+	+	+

Note: + = Positive/ Present; - = Negative/Absent.

Antioxidant activity

In 2, 2-diphenyl-1-picrylhydrazyl radical scavenging assay, antioxidant activity values are presented in (Table 2). Fig. 1 shows the dose response curve of three colours (pink, yellow and white) flower of *C. morifolium*. In case of five different concentrations (20, 40, 60, 80 and 100 mg/ml) the % scavenging activity of pink flower was gradually 89.42%, 90.78%, 91.28%, 91.98% and 92.03% and in case of yellow flower's % of scavenging activity was 26.56%, 36.79%, 44.05%, 50.78% and 60.83% and in case of white flower's % of scavenging activity was 91.98%, 92.93%, 93.27%, 93.45% and 93.62%.Where IC_{50} value of pink, yellow and white flower of chrysanthemum are respectively 11.00 mg/ml, 40.00 mg/ml and 10.00mg/ml. IC_{50} value of the positive control ascorbic acid was 11.50 mg/ml and.

These results significantly different ($P<0.05$) according to studied ANOVA (Table 3). Here, pink and white flower showed comparatively lower IC_{50} value (11mg/ml and 10mg/ml) than yellow flower of chrysanthemum (40.00 mg/ml). It is mentioned that lower IC_{50} value indicates highest antioxidant activity, and higher is lowest activity. So, this result shows that white flower contains higher antioxidant activity than pink and yellow flower.

Statistical analysis showed that the free radical scavenging power increased significantly (P<0.05) with increasing amounts of the extract.

It was very interested that white and pink flower of chrysanthemum showed better result than positive control ascorbic acid.

Table 2. DPPH radical scavenging activity of three colours (pink, yellow and white) flower of *C. morifolium* comparing with ascorbic acid.

Name of sample	IC_{50} (mg/ml)
Ascorbic acid	11.50
White flower	10.00
Pink flower	11.00
Yellow flower	40.00

[Note: IC_{50} value, the concentration of sample required for 50% scavenging of DPPH].

Discussion

The study was conducted to investigate the phytochemical analysis and antioxidant activity of three flower colours (pink, yellow and white) *Chrysanthemum morifolium*. The presence of alkaloid, flavonoids, glycoside, phenol and terpenoid in pink flowers where as saponin and tannin and were absent in pink flowers. Furthermore, alkaloid flavonoids, glycoside, saponin, tannin, terpenoid and phenol were present in white flowers. On the other hand, alkaloid flavonoids, glycoside, terpenoid and phenol in yellow flower of chrysanthemum where as saponin and tannin were absent. So, for comparing between pink, white, yellow flower, white flower of chrysanthemum was found good for biological activities and antioxidant properties than pink and yellow flower. According to the earlier reports as mentioned secondary metabolites have many therapeutic values, it can be said that both of pink, white and yellow flower of chrysanthemum can play an important role in herbal medicinal purposes.

Table 3. Statistical analysis (ANOVA).

Source of variation	df	SS	MS	F	Comment
Sample	3	17195.37	5731.789	216.5655	*
Concentration	4	674.2239	168.556	6.368589	*
Replication	1	0.921123	0.921123	0.034803	Ns
Error	31	820.4698	26.46677		
Total	39	18690.98			
Variables					Mean data
		Sample			
White flower					93.05[a]
Pink flower					91.098[a]
Ascorbic acid					90.786[a]
Yellow flower					43.802[b]
LSD					5.29
		Concentration			
20 $mgml^{-1}$					73.57125a
40 $mgml^{-1}$					77.2925a
60 $mgml^{-1}$					79.87625a
80 $mgml^{-1}$					82.0075a
100 $mgml^{-1}$					85.66375a
LSD					5.25

ns=not-significant, *=significant (P<0.05).

Lee *et al.* (2003) worked on a new anti-HIV flavonoid glucuronide from *Chrysanthemum morifolium*. A new flavonoid glucuronide, apigenin 7-O-beta-D-(4'-caffeoyl) glucuronide (1), and the known compound, apigenin 7-O-beta-D-glucurnoide, were isolated from the flowers of *C. morifolium*, Lin *et al.* (2009) identified the phenolic components of *C. morifolium* flower and Ying *et al.* (2014) worked on special effect of ionic liquids on the extraction of flavonoid glycosides from *C. morifolium* by microwave assistance. The main components were identified flavonoid glycosides, including three luteolin glycosides, three apigenin glycosids, three kaempferide glycosides and one acacetin glycoside.

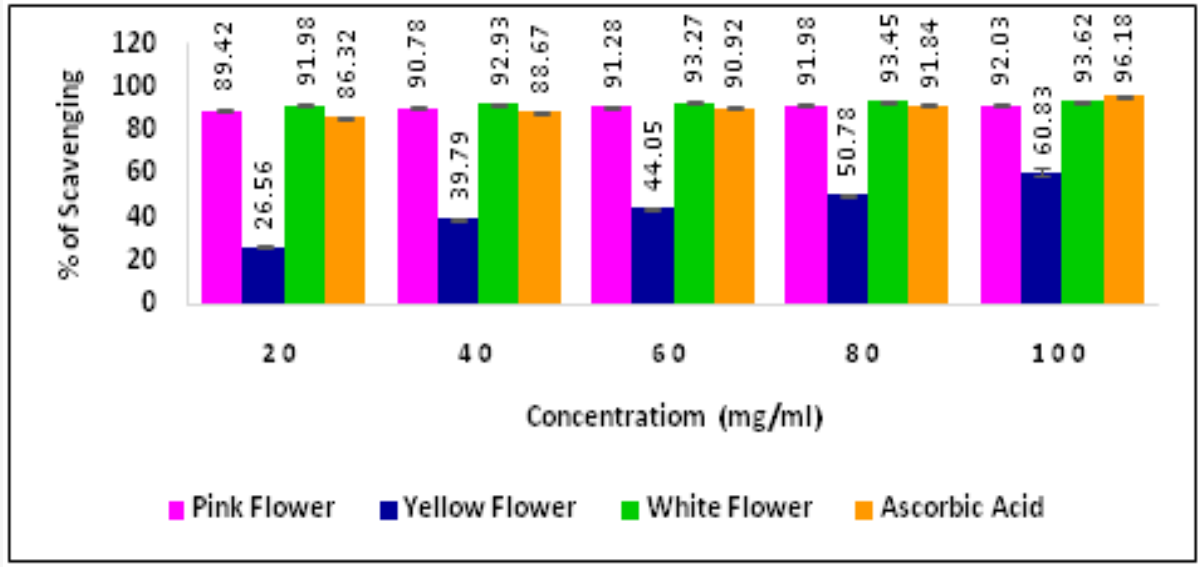

Fig. 1. DPPH radical scavenging activity of methanol extract of pink, yellow and white flower of chrysanthemum and positive control ascorbic acid.

In our investigation, the antioxidant activity of pink, yellow and white flower of chrysanthemum were examined. Here, DPPH free radical scavenging assay was used to evaluate antioxidant activity.

These three flowers showed significant antioxidant activity in a concentration dependent manner through the scavenging of 2, 2-diphenyl-1-picrylhydrazyl radicals. Where IC_{50} value of methanol extract of pink, yellow and white flower of chrysanthemum are respectively 11.00 mg/ml, 40.00 mg/ml and 10.00 mg/ml. The IC_{50} value of standard sample (ascorbic acid) was 11.50 mg/ml. Total antioxidant activity of the studied sample methanol extracts increased with increasing concentration of the extracts indicating the potential of sample antioxidants. In our result the IC_{50} value was in the order of white flower< pink flower< yellow flower. Lower IC_{50} value indicates highest antioxidant activity where higher are lowest activity.

The result showed that the IC_{50} value was highest, 40.00 mg/ml for yellow flower and lowest 10.00 mg/ml forwhite flower. The white flower which shows higher antioxidant activity than yellow and pink color flower which are significantly different ($P<0.05$) according to studied ANOVA. However, this results are supported by the phytochemical analysis of chrysanthemum.

Here, the studied sample showed the presence of alkaloid, terpenoid, phenol, flavonoid, anthraquinone. From the previous reports it is noticed that mentioned phytochemicals have antioxidant properties. As for example, Adnan *et al.* (2013), Planta Medica (2004), Foti (2007), Pietta (2000), Plumb *et al.* (1999), Grassmann (2005) reported that alkaloid, saponin, phenol, flavonoid, glycoside, terpenoid (respectively) have antioxidant properties. So, due to the presence of examined phytochemicals among the three colours flowers showed significant antioxidant activity.

In this results, white flowercontains high amount of those biochemicals than others two colours Since, in this experiment quantitative test had not be done however there are reports, where it showed that white flower contains high antioxidant compound than pink and yellow flower of chrysanthemum.

Waang *et al.* (2001) have found the aqueous chrysanthemum flower extract inhibited the production of free radicals and lipid peroxidation induced by free radicals in the heart and cerebral homogenate of rats. Kim *et al.* (2005) identified two new dicaffeoylquinic acids, 3, 5-dicaffeoyl- epi-quinic acid (1) and 1,3-dicaffeoyl- epi-quinic acid (2), were isolated from *Chrysanthemum morifolium* Ramat together with six known dicaffeoylquinic acid derivatives and three flavonoids.

The structures of the new compounds were elucidated using of spectroscopic methods. These compounds were assessed for antioxidant activities in the DPPH radical and superoxide anion radical scavenging systems.

Although different scientists have showed different result in antioxidant activity of *C. morifolium,* but there is no such reports where they showed the difference among three colours (pink, yellow and white) of chrysanthemum from where it can be compared among our results with them.

Conclusion

From the above discussion, it can be concluded that as chrysanthemum flower can be used to cure some common and other various diseases. Qualitative phytochemical screening and nutraceutical like antioxidant activity of chrysanthemum suggests that we can use chrysanthemum products as herbal medicine and with the help of the indigenous knowledge.

We can avoid production and import of many synthetic medicines and also can boost our economy by exporting our products because the current inorganic drugs in the market have several side effects and an effective means to sustain is still a challenge.

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