academicJournals

Vol. 7(36), pp. 2657-2661, 25 September, 2013
DOI: 10.5897/JMPR12.520
ISSN 1996-0875 ©2012 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

In vitro antioxidant and antimicrobial activity of anthotaxy extracts from *Dendranthema morifolium* (Ramat.) Tzvel. and *Chrysanthemum indicum* L.

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Accepted 4 August, 2013

***Dendranthema morifolium* (Ramat.) Tzvel. and *Chrysanthemum indicum* L. are two traditional Chinese medicines, which were often obscure in prescriptions. In this study, the aqueous and ethanolic anthotaxy extracts from *D. morifolium* (Ramat.) Tzvel. and *C. indicum* L., were evaluated for their antioxidant and antimicrobial properties. Ethanolic extracts showed higher contents of both total phenolics and flavonoids than aqueous extracts. The total phenolics and flavonoids contents in both aqueous and ethanolic extracts were in the order of *D. morifolium* (Ramat.) Tzvel. > *C. indicum* L. Using 1,1-diphenylpicrylhydrazyl (DPPH) assays, the concentrations providing 50% inhibition (IC_{50}) values of aqueous extracts from *D. morifolium* (Ramat.) Tzvel. and *C. indicum* L. were 2.26 and 2.93 mg/ml, respectively, whereas the IC_{50} values of ethanolic extracts were 0.38 and 1.34 mg/ml, respectively. In sum, the antioxidant activities of ethanolic extracts from both *D. morifolium* (Ramat.) Tzvel. and *C. indicum* L. were better than aqueous extracts, and in the order of *D. morifolium* (Ramat.) Tzvel. > *C. indicum* L. The ethanolic extracts exhibited moderate antimicrobial activities, whereas the aqueous extracts showed poor antimicrobial properties in our test system.**

Key words: *Dendranthema morifolium* (Ramat.) Tzvel., *Chrysanthemum indicum* L., antioxidant activity, antimicrobial activity.

INTRODUCTION

Antioxidants have been widely used as food additives to provide protection against oxidative degradation of food by free radicals. In order to prolong the storage stability of food, synthetic antioxidants are used for industrial processing. But according to toxicologists and nutritionists, the side effects of some synthetic antioxidants used in food processing such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have already been documented (Rajaei et al., 2010). For this reason, governmental authorities and consumers are concerned about the safety of the food and also about the potential affects of synthetic additives on health. Recently, numerous reports have described antioxidants and compounds with radical-scavenging activity present in herbs, fruits, vegetables and cereals extracts (Gray et al., 2002; Nuutila et al., 2003; Hou et al.,

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2005).

In the last few years, due to the misuse of antibiotics and an increasing incidence of immuno-deficiency-related diseases, the development of microbial drug resistance more and more became a pressing problem. Recently, phytochemicals with antimicrobial potential have been extensively explored to identify components for possible medical applications (Grayer and Harborne, 1994). Historically, especially in China, traditional Chinese medicines (TCMs) have played an important role in clinical therapy because of their high pharmacological activity, low toxicity and rare complication (Wen et al., 1993).

In recent years, more and more interests have been re-attracted in this field. *Dendranthema morifolium* (ramat.) tzvel. (DM) is a Chinese herb which has been used as a sudorific, antipyretic and antidote medicine (Chai et al., 2008). *Flos Chrysanthemi Indici*, anthotaxy of *Chrysanthemum indicum* L. (Asteraceae) is used as a heat-clearing and detoxication herb (Shen et al., 2004). But the two flowers are often obscure in prescriptions. The present study was undertaken to investigate the comparative antioxidant and antimicrobial activities of their aqueous and ethanolic extracts to provide scientific evidence for further application of them as resource for medicinal and nutritional purposes.

MATERIALS AND METHODS

Plant

Anthotaxies of *D. morifolium* (Ramat.) Tzvel. and *C. indicum* L. were collected in Anhui Province and Sichuan Province (P.R. China) in 2010, respectively. To generate aqueous extracts, 10 g of powdered samples were soaked in 100 ml boiling distilled water and stirred for 3 h. The filtrate was stored at -4°C. To obtain the ethanolic extracts, 10 g of powdered samples were soaked in 100 ml 80% ethanol, refluxed at 80°C for 3 h. The filtrate was stored at -4°C.

Determination of total phenolics and total flavonoids

The content of total phenolic phytochemicals was measured using the Folin-Ciocalteu method. Briefly, 1 ml of appropriately diluted samples or a standard solution of gallic acid (Sigma-Aldrich) was added to 1 ml of Folin-Ciocalteu phenol reagent (1 N), mixed by shaking and allowed to react for 5 min. Then, 2 ml of 20% Na_2CO_3 solution was added and allowed to react for 10 min at room temperature in comparison to gallic acid as standard. Absorbance was measured at 730 nm using a spectrophotometer (AWARENESS). The total phenolic content was expressed in milligrams of gallic acid equivalents (GAE) per 1 g of fresh herb. The flavonoid contents were measured by a colorimetric assay. Each 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 ml standard solutions of rutin (Sigma-Aldrich) or 0.3 ml extracts were mixed with 0.5 ml of 5% (w/v) sodium nitrite. After incubation for 6 min, 0.5 ml of 10% (w/v) aluminium nitrate was added to the mixture. Then 2.5 ml of 5% sodium hydroxide was added to the mixture for further 6 min, followed by addition of distilled water to a final volume of 10 ml. Absorbance was read at 500 nm against blank, and flavonoid content was expressed as mg rutin equivalents per 1 g of fresh herb. Samples were analyzed in triplicate.

DPPH radical scavenging assay

The stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used as reagent (Amarowicz et al., 2004). Each 100 µl of samples in a concentration range from 0.390625 to 12.5 mg/ml were mixed with 1.4 ml of ethanol and added to 1 ml 0.004% (w/v) DPPH (Sigma-Aldrich) in ethanol. The mixture was vigorously shaken and then immediately placed in a spectrophotometer to monitor the decrease in absorbance at 517 nm. Monitoring was continued for 70 min until the reaction reached a plateau. Ethanol was used as negative control. Ascorbic acid (Sigma-Aldrich), a stable antioxidant, was used as positive control. The radical-scavenging activities were expressed as percentage inhibition of DPPH and calculated according to the formula:

Inhibition percentage (Ip) = [(AB - AA) / AB] × 100 (Yen and Duh, 1994)

Where AB and AA are the absorbance values of the blank sample and test samples after 70 min, respectively. Samples were analyzed in triplicate.

Antimicrobial activity

Microorganisms

Bacillus subtilis (Bs), *Staphylococcus aureus* (Sa), *Staphylococcus epidermidis* (Se), *Escherichia coli* (Ec), *Pseudomonas aeruginosa* (Pa) and *Candida albican* (Ca) were test strains derived from type culture collection (ATCC, U.S.A.; NCTC, UK; DSM, Germany). The microorganisms were cultured overnight on agar and aerobically incubated at 37°C.

Broth microdilution method

Serial doubling dilutions of the extracts were prepared in a 96-well microtiter plate ranging from 0.7813 to 100 mg/ml. Each dilution (100 µl) was dispensed into the wells, then inoculated with 100 µl of the bacterial suspension. The final concentration of each strain was adjusted to 10^5 to 10^6 CFU/ml. The MIC is defined as the lowest concentration of the extracts at which the microorganism being tested does not demonstrate visible growth. The minimal bactericidal concentration (MBC) is the lowest concentration without colony growth on the agar plates, determined by seeding 10 µl from each well on a plate which was then incubated for further 24 h at 37°C. Streptomycin (Sigma) and amphotericin (Sigma) were used as positive control. All experiments were performed in triplicate.

RESULTS

Total phenolics and flavonoids

Polyphenols are bioactive compounds involved in the cellular defense against deleterious oxidative damage, at least in part, due to their antioxidant properties (Fresco et al., 2006). Because purified phenolic compounds are difficult to obtain and extracts sometimes have better

Table 1. The total phenolics and total flavonoids of *D. morifolium* (ramat.) tzvel. and *C. indicum* L. aqueous and ethanolic extracts.

Parameter	Total phenolics (mgGAE/g)		Total flavonoids (mg/g)	
	Aqueous extracts	Ethanolic extracts	Aqueous extracts	Ethanolic extracts
D. morifolium (ramat.) tzvel.	72.73±3.73	99.09±5.84	69.48±4.29	82.09±4.68
C. indicum L.	41.81±3.57	54.40±3.86	37.37±4.31	42.78±4.26

Table 2. The IC_{50} values of *D. morifolium* (ramat.) tzvel. and *C. indicum* L. aqueous and ethanolic extracts using DPPH assay.

Parameter	Aqueous extracts (mg/ml)	Ethanolic extracts (mg/ml)
D. morifolium (ramat.) tzvel.	2.26	0.38
C. indicum L.	2.93	1.34

antioxidant activities than those of pure molecules, there is a growing interest for the use of plant extracts (Calliste et al., 2005; Barreira et al., 2008). The amount of total phenolics in the aqueous and ethanolic extracts are shown in Table 1. The contents of total phenolics of aqueous anthotaxies extracts from *D. morifolium* (Ramat.) Tzvel. and *C. indicum* L. were 72.73 ± 3.67 and 41.81 ± 3.57 mgGAE/g, respectively, whereas the contents in ethanolic extracts were 99.09 ± 5.84 and 54.40 ± 3.86 mgGAE/g, respectively. In general, the ethanolic extracts contained higher amounts of total phenolics than aqueous extracts, and in *D. morifolium* (Ramat.) Tzvel. extracts was also higher than in *C. indicum* L. The amount of total flavonoids from *D. morifolium* (Ramat.) Tzvel. and *C. indicum* L. aqueous extracts were 69.48 ± 4.29 and 37.37 ± 4.31 mg/g, respectively, whereas those of ethanolic extracts were 82.09 ± 4.68 and 42.78 ± 4.26 mg/g, respectively (Table 1). In general, the ethanolic extracts contained higher amounts of total flavonoids than aqueous extracts in the order: *D. morifolium* (Ramat.) Tzvel. > *C. indicum* L.

Free radical-scavenging activity: DPPH assay

The free radical scavenging activities of *D. morifolium* (Ramat.) Tzvel. and *C. indicum* L. aqueous extracts (3.125 mg/ml) were 80.57 ± 4.87 and 62.18 ± 4.26%, respectively (Figure 1), whereas at the same concentration, free radical scavenging activities of ethanolic extracts were 94.61 ± 4.15 and 89.18 ± 4.95%, respectively (Figure 2). The antioxidant activities of the extracts were concentration-dependent. The scavenging effects of all extracts increased with increasing concentrations. The 50% inhibition concentrations (IC_{50}) values of aqueous extracts from *D. morifolium* (Ramat.) Tzvel. and *C. indicum* L. were 2.26 and 2.93 mg/ml, respectively, whereas the IC_{50} values of ethanolic extracts were 0.38 and 1.34 mg/ml, respectively (Table 2). When compared to ascorbic acid (IC_{50} = 1.05 mg/ml), only ethanolic extracts from *D. morifolium* (Ramat.) Tzvel. was better than it, all other extracts were less effective. In summary, the antioxidant activities of ethanolic extracts were better than those of aqueous extracts, and *D. morifolium* (Ramat.) Tzvel. extracts processed better antioxidant activities than *C. indicum* L.

Antimicrobial activity

The results of antimicrobial activities testing for *D. morifolium* (Ramat.) Tzvel. and *C. indicum* L. ethanolic anthotaxies extracts are given in Table 3. Six microorganisms (three gram-positive bacteria, two gram-negative bacteria and one fungi) were tested. The ethanolic extracts of *D. morifolium* (Ramat.) Tzvel. possessed much better antimicrobial activities than *C. indicum* L. extracts. *S. aureus* was the most sensitive bacterium in our test panel. The MIC and MBC values for the ethanolic extracts from *D. morifolium* (Ramat.) Tzvel. and *C. indicum* L. were 0.781 and 3.13 mg/ml, respectively. The MBC values were 6.25 and 12.5 mg/ml, respectively. All extracts were less effective when compared to positive control. In our test system, the aqueous extracts from both *D. morifolium* (Ramat.) and *C. indicum* L. all showed poor antimicrobial activity with MICs and MBCs all higher than 25 mg/ml.

DISCUSSION

In the recent years, the antioxidant and antimicrobial may be useful in retarding oxidative deterioration of food materials especially those with high lipid content. It is well known that reactive oxygen species (ROS) formed *in vivo*, such as superoxide anion, hydroxyl radical and hydrogen peroxide, are highly reactive and potentially damaging transient chemical species (Nain et al., 2011). Reactive oxygen species, causing damage to DNA, proteins and

Table 3. Antimicrobial activity of *D. morifolium* (ramat.) tzvel. and *C. indicum* L. ethanolic extracts.

Microbial	*D. morifolium* (ramat.) tzvel. (mg/ml)		*C. indicum* L. (mg/ml)		Control (µg/ml)	
	MIC	MBC	MIC	MBC	MIC	MBC
Bs	1.56	50	3.13	>50	31.25[a]	125[a]
Sa	0.781	6.25	3.13	12.5	31.25[a]	>125[a]
Se	12.5	12.5	12.5	12.5	7.81[a]	125[a]
Ec	12.5	25	12.5	12.5	62.5[a]	>125[a]
Pa	3.13	25	6.25	25	125[a]	>125[a]
Ca	3.13	50	6.25	50	0.20[b]	0.20[b]

a: Streptomycin; b: amphotericin B.

lipids, have been associated with carcinogenesis, coronary heart disease, and many other health problems (Sasidharan and Menon, 2010). The possibe ways to fight these uncurable diseases is to improve our body's transformation due to antioxidant defenses. High consumption of plants, fruits and vegetables has been associated with a lowered incidence of such degenerative or incurable diseases (Bajpai et al., 2009). The functional bioactivity of plant extracts, essential oil in general, depends on the presence of compounds such as plyphenols, carotenoids and cholorophyll (Shah and Hossain, 2011). Plants can contribute in this area primarily due to the antioxidant activity of polyphenolic compounds (Hossain and Shah, 2011).

The phenolic compounds such as flavonoids and terpenoids are the dominant antioxidants that exhibit scavenging efficiency on free radicals and reactive oxygen species are numerous and widely distributed in the plant kingdom. In our investigation, ethanolic extracts from *D. morifolium* (Ramat.) Tzvel. and *C. indicum* L. showed higher contents of both total phenolics and flavonoids than aqueous extracts and *D. morifolium* (Ramat.) Tzvel. extracts contained higher contents of both phenolics and flavonoids than *C. indicum* L. extracts. The stable DPPH is a widely used method to evaluate antioxidant activities in a relatively short time compared to other methods. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The decrease in absorbance of DPPH caused by antioxidants is due to the reaction between antioxidant molecules and radical, which results in the scavenging of the radical by hydrogen donation. This is visualized as a discoloration from purple to yellow. In our test, both aqueous and ethanolic extracts from *D. morifolium* (Ramat.) Tzvel. possessed better antioxidant acitvities than *C. indicum* L.

The *D. morifolium* (Ramat.) Tzvel. ethanolic extracts possessed much better antimicrobial activities than *C. indicum* L., however, aqueous extracts showed poor antimicrobial activities in our test system. The antimicrobial effect of extracts against these organisms may be due to presence of the bioactive compounds of plants like falvanoids, alkaloids, saponin and tannins. Further work can be carried on the isolation procedure for finding out the exact moiety responsible for the biological activity.

Conclusion

The results in this *in vitro* study could support the use of *D. morifolium* (Ramat.) Tzvel. and *C. indicum* L. by traditional healers to treat various infective diseases, furthermore, *D. morifolium* (Ramat.) Tzvel. extracts possessed both better antioxidant and antimicrobial activities than *C. indicum* L. extracts, so they should not be confused for prescriptions.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial supports by postdoctoral science-research developmental foundation of Heilongjiang province (LBH-Q11183).

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