Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading

A comparative study on the antioxidant activity of methanolic leaf extracts of *Ficus religiosa* L, *Chromolaena odorata* (L.) *King & Rabinson*, *Cynodon dactylon* (L.) Pers. and *Tridax procumbens* L.

Melinda Krishanti P[1], Xavier Rathinam[1*], Marimuthu Kasi[1], Diwakar Ayyalu[1], Ramanathan Surash[2], Kathiresan Sadasivam[1], Sreeramanan Subramaniam[3]

[1]*Department of Biotechnology, Faculty of Applied Sciences, AIMST University, Semeling, 08100 Bedong, Kedah Darul Aman, Malaysia*
[2]*Institute for Drug Reasech, Universiti Sains Malaysia, 11800 Penang, Malaysia*
[3]*School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia*

ARTICLE INFO

Article history:
Received 14 March 2010
Received in revised form 16 April 2010
Accepted 30 April 2010
Available online 20 May 2010

Keywords:
Chromolaena odorata
Antioxidant activity
Phytochemical analysis

ABSTRACT

Objective: To compare the antioxidative effects of the methanolic leaf extracts of *Ficus religiosa* (*F. religiosa*), *Chromolaena odorata* (*C. odorata*), *Cynodon dactylon* (*C. dactylon*) and *Tridax procumbens* (*T. procumbens*) as well as the contents of antioxidants in the extracts. **Methods:** Total phenol and total flavanoid contents were measured according to the standard procedures. The total antioxidant capacity was determined using the phosphomolybdenum method. Reducing power was determined by the potassium ferricyanide reducing method. The free radical scavenging activity was measured by 2,2′-Diphenyl-1-picrylhydrazyl (DPPH) assay. **Results:** Quantitative phytochemical analysis of total phenol content showed that *C. odorata* had the highest content of phenolic compounds significantly followed by *F. religiosa*, *T. procumbens* and *C .dactylon*. As for the total flavanoids content, *F. religiosa* had the highest content, followed by *C. odorata*, *T. procumbens* and *C. dactylon*. Study on the total antioxidant capacity revealed that *F. religiosa*, *C. dactylon* and *C. odorata* showed higher total antioxidant capacity. *T. procumbens* showed the lowest capacity. Meanwhile, *T. procumbens* and *C. odorata* have the highest reducing power activity followed by *F. religiosa* and *C. dactylon*. The results of DPPH radical scavenging activity indicated that *T. procumbens* induced the largest elevation as the concentration of its extract increased, followed by *C. odorata* and *F. religiosa* and *C. dactylon*. **Conclusions:** The present study demonstrates the antioxidative capacity of all the four plant species. Of all the plants, *C. odorata*, a perennial weed plant showed potentially a high antioxidant activity, with higher phenolic and flavonoids contents. The data suggest that *C. odorata* can be best utilized in developing bioantioxidants.

1. Introduction

Reactive oxygen species (ROS) are considered to involve in the pathogenesis of many diseases, including cancer, diabetes and atherosclerosis[1]. Synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) are powerful, however, they are proved to be toxic to humans so that they are just for industrial use[2]. Therefore, it is urgent to find natural antioxidants. The antioxidative effects of plants are mainly due to the presence of phenolic compounds such as flavonoids, phenolic acids, tannins and phenolic diterpenes that may exist in all parts of plants including fruits, vegetables, nuts, seeds, leaves, roots and barks[3]. *Ficus religiosa* L. (Fam, Moraceae)(*F. religiosa*) is regarded as a sacred plant distributing in most of the Asian countries. *Chromolaena odorata* (L.) King and Robinson (Fam, Asteraceae) (*C. odorata*) and *Tridax procumbens* L. (Fam, Asteraceae) (*T. procumbens*) have been considered as weed plants with potential medicinal values. *Cynodon dactylon* (L.) Pers. (Fam, Poaceae) (*C. dactylon*) is a kind of perennial grass, which its leaf, root and rhizome have been used in folk medicine of different countries. The main objective of this study is to compare the antioxidant activity of these indigenous plants and its correlation with the contents of antioxidants in the plants.

*Corresponding author: Dr. Xavier Rathinam, Department of Biotechnology, Faculty of Applied Sciences, AIMST University, Jalan Bedong Semeling, 08100, Bedong, Kedah Darul Aman, Malaysia.
E-mail: rxavier77@yahoo.com

2. Materials and methods

2.1. Materials

All the chemicals and reagents used in this study were ACS grade. Leaf samples of *F. religiosa*, *C. odorata*, *C. dactylon* and *T. procumbens* were collected in Sungai Petani, Kedah Darul Aman, Malaysia, in April 2009. The plants were identified and authenticated by a botanist from the Faculty of Applied Sciences, AIMST University, Kedah Darul Aman, Malysia.

2.2. Preparation of plant extracts

Leaves free from microbial infection and of uniform maturity were collected. Firstly, leaves were washed with clean running tap water to remove the contaminating foreign particles. Then leaves were cut into small pieces and oven dried at 45 ℃ for 10 days for complete drying. The leaves were powdered using a heavy duty blender. The powders were extracted with methanol according to the maceration method and the extracts were filtered by Whatman No.1 filter paper. The filtrates were concentrated in a rotary evaporator at 40 ℃. The concentrated extracts were oven dried at 40 ℃for 4 days and then freeze dried for 48 hours. The freeze dried extracts were stored at -20 ℃ until use.

2.3. Phytochemical analysis

2.3.1. Total phenol content assay

Phenolic contents were estimated by colorimetric assay[4] with gallic acid as a standard. Crude methanol extracts (0.5 mg/mL) were prepared. Then, 1 mL of extracts mixed thoroughly with 1 mL of Folin-Ciocalteu reagent. After 3 min, 3 mL of Na_2CO_3 (2%) was added, then the mixture was allowed to stand for 2 hours at room temperature with intermittent shaking. Absorbance was measured at 725 nm. All tests were performed in triplicate. The concentration of the total phenolic compounds in extract was determined as gram of gallic acid equivalent.

2.3.2. Total flavonoid content assay

The content of total flavonoid was measured spectrophotometrically as previously described[5]. The method based on the formation of a complex flavonoid-aluminium, having absorbance maximum at 430 nm. Cathecin was used to make a calibration curve. 0.5 mL of extract solution was mixed with 2 mL distilled water and 0.15 mL of 15% $NaNO_2$. The mixture was incubated for 6 minutes and then 0.15 mL of 10% $AlCl_3$ was added. After 6 minutes, 2 mL of 4% NaOH solution was added. Distilled water was then added to bring the sample to 5 mL. Before measuring the absorbance at 510 nm, the mixture was allowed to stand for 15 min. The flavonoids content was expressed as cathecin equivalents in mg/g extract.

2.4. Measurement of total antioxidant capacity

The total antioxidant capacity of the methanolic extract was determined using the phosphomolybdenum method[6]. First, 0.3 mL of the extract was mixed with 1 mL of reagent solution. The reaction mixture was incubated at 95 ℃ for 30 minutes. After cooling at room temperature, the absorbance of the mixture was measured at 695 nm against a blank. Ascorbic acid was used as a standard. The antioxidant activity was expressed as μg/mL ascorbic acid equivalents (AAE).

2.5. Detection of reducing power activity

Reducing power of extract was determined according to the previous method[7]. Plant extracts were dissolved in methanol (2 mg/mL) and mixed with 2.5 mL phosphate buffer (pH 6.6, 200 mM) and 2.5 mL of potassium ferricyanide. The mixture was incubated at 50 ℃ for 20 minutes. Then, added 2.5 mL of 10% tricholoroacetic acid and centrifuged at 3 000 rpm for 10 minutes. The supernatant obtained (2.5 mL) was mixed with distilled water (2.5 mL) and 0.1% ferric chloride (0.5 mL), and the absorbance was measured at 700 nm. Increased absorbance indicates increased reducing power activity.

2.6. Measurement of DPPH free radical scavenging activity

Reduction of 2,2′-Diphenyl-1-picrylhydrazyl (DPPH) radical to diphenylpicryl hydrazine by the plant extracts was measured spectrophotometrically at 517 nm by the method previously described[8]. BHT with the same concentrations was used as standard and also for comparison. All tests were performed in triplicates. The antioxidant activity was expressed as a percentage of scavenging of DPPH and is calculated using the equation of $[(A_0-A_1)/A_0]\times100$ where A_0 was the absorbance of the control and A_1 was the absorbance of the sample.

2.7. Statistical analysis

All the assays were carried out in triplicates. Experimental results were expressed as mean±standard deviation (SD) of three parallel measurements. The differences between the extracts were analysed via one-way analysis of variance (ANOVA) using SPSS version 11.

3. Results

The four plants are locally available to treat various disease related with oxidative damage, so in our study we evaluated the antioxidative properties of the methanolic leaf extracts. Quantitative phytochemical analysis of total phenolic content showed that *C. odorata* had the highest content of phenolic compounds significantly [(455.55±4.59) μg/mg GAE] followed by *F. religiosa* [(235.00±4.41) μg/mg GAE], *T. procumbens* [(147.78±2.09) μg/mg GAE] and *C. dactylon* [(137.50 ±3.82) μg/mg GAE]. The amount of flavanoids in the plant extracts can directly correlate with its antioxidant activity. In our data, *F. religiosa* showed the highest content of total flavonoids [(93.67±0.60) mg/mL CE], followed by *C. odorata* [(62.83±0.30) mg/mL CE], *T. procumbens* [(41.30±0.30) mg/mL CE] and *C. dactylon* [(35.80±0.30) mg/mL CE].

Study on the total antioxidant capacity revealed that *F. religiosa*, *C. dactylon* and *C. odorata* had higher total antioxidant capacity with values of [(10.50±0.06) μg/mL AAE], [(10.48±0.03) μg/mL AAE] and [(10.39±0.03) μg/mL AAE], respectively. *T. procumbens* showed the lowest total antioxidant capacity [(9.00±0.04) μg/mL].

The reducing capacity is an important indicator of its potential antixidant activity, and this test based on the ability of a compound to convert Fe^{3+} into Fe^{2+}. Increased absorbance indicates increased reducing power activity. It showed *T. procumbens* (1.336±0.03) and *C. odorata* (1.297± 0.06) had the highest reducing power activity followed by *F. religiosa* (0.788 ±0.02) and *C. dactylon* (0.726±0.02).

The results of DPPH radical scavenging activity indicate that for all the extracts, the inhibition increased with the elevated concentration using BHT, a commercial antioxidant as the standard. Overall, *T. procumbens* showed the highest increase in percentage of scavenging activity followed by *C. odorata* and *F. religiosa*. *C. dactylon* had the least scavenging activity but still showed an improvement as the concentration of its extract increased (Figure 1). Surprisingly, despite the lesser amounts of phenolics and flavanoids as well as lower total antioxidant capacity, *T. procumbens* showed a higher DPPH activity. This paradox might be due to different types of antioxdative compounds in the extract and the solvent used for the extraction.

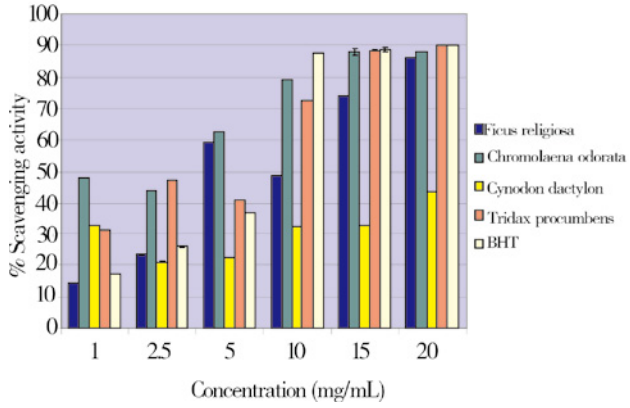

Figure 1. DPPH radical scavenging activity of the methanolic extracts of different plant species.Values are expressed as mean±SD.

4. Discussion

Free radicals are thought to play an important role in many disease such as chronic and degenerative diseases including aging, coronary heart disease, inflammation, stroke, diabetes mellitus and cancer[9]. Plant-based alternatives are more viable and safer compared to the synthetic products. Phenolic compounds, including flavanoids are considered as the most important antioxidative components of plant materials because of the positive correlation between the concentration of plant phenolics and its total antioxidant capacity[10]. Our study justified the use of the much neglected and unwanted weed plants such as *C. odorata*, *C. dactylon* and *T. procumbens* as human therapeutics. In general the *Ficus* spp. and *C. dactylan* are known medicinal plants with a variety of therapeutic potential. This study assumes the importance in identifying the potential antioxidant capacities of weed plants which can be alternatives.

The higher amount of phenolics in *C. odorata* suggests that this plant can be harnessed in the development of phytoantioxidants, otherwise a weed plant that smothers the growth of other vegetation in the vicinity. Despite the fact that *F. religiosa* is rich in total flavonoids, *C. odorata* had a comparable amount of flavanoids. Several reports indicated the presence of higher amounts of phenols in the plants belonging to the family Moraceae, particularly in the genus *Ficus*[11]. The results of DPPH assay, reducing power activity and total antioxidant activity, consistently showed the antioxidant potential of *C. odorata*.

In conclusion, the present study demonstrates the antioxidative capacity of all the four plant species. Of all the plants, *C. odorata* is found to have a consistent correlation between its phenolic compounds and its free radical scavenging activities. It is a fast-growing perennial, invasive weed and an aggressive competitor that occupies different types of lands and forms dense strands that prevents the establishment of other flora. The potential antioxidant activity of *C. odorata* can be best utilized as a therapeutic to protect the human system in managing the reactive oxygen species. The specific compounds contributing to the antioxidant activities were not known. The future studies may be directed to identify the specific compounds using High Performance Liquid Chromatography (HPLC). In addition, different extraction methods could be applied such as aqueous, chloroform, petroleum ether and crude hot water extract to extract different compounds. A more profound knowledge of the compounds present and their properties will allow application of the extracts in the food and pharmaceutical industry.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1]Moskovitz J, Yim MB, Chock PB. Free radicals and disease. *Arch Biochem Biophy* 2002; **397**: 354-9.
[2]Safer AM, Al-Naghamish, AJ.Hepatotoxicity induced by the anti-oxidant food additive butylated hydroxytoluene (BHT) in rats: An electron microscopical study. *Histol Histopathol* 1999; **14**: 391-406.
[3]Ayoola GA, Folawewo AD, Adesegun SA, Abioro OO, Adepoju-Bello AA, Coker HAB. Phytochemical and antioxidant screening of same plants of apocynacea from South West Nigeria. *Afr J plant Sci* 2008; **2**(9):124-8.
[4]Slinkard K, Singleton VL. Total phenol analysis: Automation and comparison with manual methods. *Am J Enol Vitic* 1977; **28**: 49-55.
[5]Djeridane A, Yousfi M, Nadjemi B, Boutassaouna D, Stocher PS. Antioxidant activity of some Algerian medicinal plant extracts containing phenolic compound. *Food Chemistry* 2006; **97**: 654-60.
[6]Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of phosphomolybdenum complex: specific application to determination of vitamin E. *Analytical Biochemistry* 1999; **269**: 337-41.
[7]Oyaizu M. Studies on product of browning reaction prepared from glucose amine. *Jap J Nutr* 1986; **44**: 307-15.
[8]Hatano T, Kagawa H, Yasuhara T, Okuta T. Two new flavonoids and other constituents in licorice roots: their relative astringency and radical scavenging effects. *Chem Pharm Bull* 1988; **36**: 1090-2097.
[9]Cheng HY, Lin TC, Yu KH, Yang CM, Lin CC. Antioxidant and free radical scavenging activities of *Terminalia chebula*. *Biol Pharm Bull* 2003; **26**:1331-5.
[10]Pellegrini N, Simonetti P, Gardana C, Brenna O, Brighenti F, Pietta P. Polyphenol content and total antioxidant activity of Vini Novelli (Young red wines). *J Agr Food Chem* 2000; **48**: 732-5.
[11]Ao C, Li A, Elzaawely AA, Xuan TD, Tawata S. Evaluation of antioxidant and antibacterial activities of *Ficus microcarpa* L. fil. extract. *Food Control* 2007; **19**(10): 940-8.