Asian Journal of Plant Sciences, 2012
ISSN 1682-3974 / DOI: 10.3923/ajps.2012.

Evaluation of Phytochemical Composition and Antibacterial Property of *Gynura procumbens* Extract

[1]N. Kaewseejan, [1,2]D. Puangpronpitag and [1]M. Nakornriab
[1]Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Mahasarakham University, Mahasarakham 44150, Thailand
[2]Faculty of Medicine, Mahasarakham University, Mahasarakham 44000, Thailand

Abstract: *Gynura procumbens* is one of the important medicinal plants in Thailand and Southeast Asia. It is usually used as a traditional medicine for the treatment of inflammation, herpes simplex virus, rashes, fever, rheumatism, kidney disease, migraines, constipation, diabetes mellitus, cancer and hypertension. The present study was to screen phytochemical compositions of ethanol extract of *Gynura procumbens* leaves such as chlorophylls, carotenoids, alkaloids, saponins, anthraquinone glycosides, volatile oils as standard methods and to evaluate the antibacterial activity by using agar well diffusion method. Phytochemical analysis revealed the contents of chlorophyll-a, b and carotenoids in ethanolic *Gynura* extract were 365.20±0.049, 132.40±0.029 and 53.20±0.034 μg g^{-1} dry weight, respectively. Moreover, the ethanolic *Gynura* extract showed the presence of alkaloids and volatile oils, whereas saponins and anthraquinone glycosides were absent. According to determination the antibacterial activity was found that *Gynura procumbens* showed as negative effects in all bacteria's tested. These results suggest that *Gynura* extract is a good natural source of bioactive compounds and that they may have beneficial health effects for consumption which may use as preliminary information and could be further studied for uses in food industry, health product, pharmaceutical and medicinal applications.

Key words: Medicinal plant, chlorophyll, carotenoid, alkaloids, agar well diffusion

INTRODUCTION

Traditional medicinal plants have been widely used as major sources of drugs in pharmaceutical industry. The research into medicinal plants with alleged folkloric use as pain relievers, anti-inflammatory agents (Jothimanivannan *et al*., 2010). Medicinal plant is any plant from which valuable drugs can be synthesized as it contains substances that can be used for medicinal purposes (Karim *et al*., 2011). The cultivation of medicinal plants on large scale is increasing in order to provide raw material for the manufacturing of herbal products. In developing countries, farmers are practicing co-cultivation of medicinal (herbal) plants to get additional income. Each parts of medicinal plants have it owns used for treatment of certain disease. Numerous examples had impressively demonstrated the innovative potential of natural compounds and their impacts on the progress of drugs discovery and development (Divya *et al*., 2011; El-Baz *et al*., 2002; Abd El-Aziz, 2011). About 30% of drugs used worldwide are based on natural products (Subhash and Vaishana, 2010). Plants have been the basis of traditional medicines throughout the world for thousands of years and continue to provide new remedies to humankind. Several authors have reviewed the beneficial uses of these plant species (Siriwatanametanon *et al*., 2010; Matkowski, 2008; Scartezzini and Speroni, 2000). Nowadays, the usage of medicinal plants in forms of traditional medicine, herbal medicine, botanical dietary supplement and so on increase considerably (Shafaei *et al*., 2011). Scientific evidence of the medicinal plants is thus needed to evaluate the safety, efficacy and quality of the herbal medicinal products. Over the years therapeutic manipulations in folk medicine and orthodox medical practices has been dependent on pharmacopoeia formulations from plants. These plants, generally called medicinal plants, have been found to contain active secondary metabolites called phytochemicals and some of them have medicinal properties, such as antioxidants (Karim *et al*., 2011; Hasani-Ranjbar *et al*., 2009). The continued investigation of the secondary plant metabolites has led to important breakthroughs in pharmacology and has helped tremendously in the development of modern pharmacotherapeutics in the world (Duru and Onyedineke, 2010). Plant products

Corresponding Author: D. Puangpronpitag, Department of Chemistry and Center for Innovation in Chemistry, Faculty of Science and Faculty of Medicine, Mahasarakham University, Mahasarakham 44150, Thailand

today symbolize safety in contrast to synthetic drugs (Gill *et al*., 2010). Therefore, discovery of new plant-based drugs without any adverse effects has been paid in the screening of plant-based drugs which are used in the traditional system of medicine.

Gynura procumbens (Compositae) is an annual evergreen shrub with a fleshy stem and purple tint. *G. procumbens* is found in various parts of Asia and is widely used in Thailand and Southeast Asia as a traditional medicine. This plant is used in the traditional treatment of various health aliments such as eruptive fevers, rash, kidney disease, migraines, constipation, hypertension, diabetes mellitus and cancer (Perry, 1980). The literature search suggests that leaves or leaves extracts of *G. procumbens* has anti-herpes simplex virus (Nawawi *et al*., 1999), antihyperglycemic (Li *et al*., 2009; Akowuah *et al*., 2002), antihyperglycaemic and antihyperlipidaemic (Zhang and Tan, 2000), anti-inflammatory (Iskander *et al*., 2002), anticarcinogenic (Agustina *et al*., 2006), blood hypertension reduction capabilities (Hoe *et al*., 2006; Kim *et al*., 2006), antiproliferative on human mesangial cell (Lee *et al*., 2007), antioxidative (Puangpronpitag *et al*., 2010; Rosidah *et al*., 2008, 2009) and anti-ulcerogenic (Mahmood *et al*., 2010) properties. The leaves of this plant are often consumed in diet and research shows that leaves contents are not having any toxic effects (Rosidah *et al*., 2009). The benefits of the traditional use of *G. procumbens* have also been supported by the isolation and identification of several possible flavonoid constituents from this plant (Akowuah *et al*., 2002). *G. procumbens* is an important tropical medicinal plant is studied mainly of its medicinal properties and toxicology of leaves or leaves extracts and there is no study so far about the phytochemical associated with this important plant.

Therefore, the present study was interested to further investigate this plant, with a view to determining the phytochemical composition of the *G. procumbens* leaf extract as well as the chlorophylls and carotenoids contents of the extract. In addition, the antibacterial activity of the plant extract was also investigated. The results of this study would be preliminary information that worth to further studied for uses in food industry, health product, pharmaceutical and medicinal applications.

MATERIALS AND METHODS

Plant material: *G. procumbens* was collected from the local area of KhonKaen Province, KhonKaen, Thailand, in February 2010. Fresh leaves (100-200 g) were air-dried and then oven dried at 60°C for 24 h. The dried leaves of *G. procumbens* samples were ground into fine powder using mortar and then the sample was stored in desiccators until uses.

Preparation of extract: One gram of oven-dried, finely powdered leaves of *G. procumbens* was extracted with 20 mL of ethanol at room temperature for 3 h. After that, the extract was filtered through Whatman No. 1 filter paper and concentrated to dryness by rotary evaporation at 35°C *in vacuo*. After freeze dried, the concentrated sample was pooled and stored in refrigerator until used for determination of antibacterial, tyrosinase inhibition and antioxidative protein damage activities. All the extractions were performed in triplicate.

Determination of chlorophyll and carotenoid contents: The contents of chlorophyll-a, b and total carotenoids of *G. procumbens* extract were determined according to the modified method (Madison and Anderson, 1963). One gram of oven dried, finely powdered leave of sample was extracted with 20 mL of 80% ethanol. The extract was filtered through Whatman No.1 filter paper. Two milliliters of extract was centrifuged at 1200 xg for 20 min and detected absorbance at 400-700 nm by UV-Vis spectrophotometer (Lamda 25, Perkin Elmer). Chlorophyll a showed the maximum absorbance at 665.2 nm, chlorophyll b at 652.4 nm and total carotenoids at 470 nm. Chlorophyll a, chlorophyll b and total carotenoids contents (μg mL^{-1}) were calculated using the following this formula and expressed as μg g^{-1} dry weight:

$$\text{Chlorophyll a} = 16.72\ (A_{665.2})\text{-}9.15\ (A_{652.4})$$

$$\text{Chlorophyll b} = 34.09\ (A_{652.4})\text{-}15.28\ (A_{665.2})$$

$$\text{Carotenoids} = [1000\ (A_{470})\text{-}1.63(\text{Chl } a)\text{-}104.96\ (\text{Chl b})]/221$$

Phytochemical analysis: The ethanolic *G. procumbens* extract was determined to phytochemical tests for plant secondary metabolites; alkaloids, saponins, anthraquinone glycosides and volatile oils using standard procedures methods as described with little modification (De *et al*., 2010; Ayoola *et al*., 2008).

Antibacterial activity: Agar well diffusion method was used in the screening antibacterial activity as described by Magaldi *et al*. (2004). Five bacteria tests; *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus cereus*, *Pseudomonas aeruginosa* and

Escherichia coli were cultured on Mueller Hinton Broth media plates at 37°C for 18 h. Bacteria were adjusted the turbidity (OD_{600} nm) to match of standard McFarland No. 0.5. The bacteria suspensions were used to inundate sterile plates containing 20 mL Mueller Hinton Agar (MHA). A well was prepared in the plates with the help of a cork-borer No. 2. The one hundred microliters of each plant solutions were placed into the inoculated agar well surface. The plates were incubated at 37°C for 18-24 h. The results were recorded by measuring the zone of inhibition surrounding the well plates indicating the presence of antibacterial activity. The sterile distilled water was used as a negative control.

Statistical analysis: In all the determinations were performed in triplicate (n = 3) and the data are express as the Mean±Standard Deviation (SD).

RESULTS

In this study, the medicinal plant *Gynura procumbens* leaves were extracted in ethanol to 14.73% yield of dried grind powder. Ethanolic *Gynura* extract was screened the preliminary phytochemical compositions such as alkaloids, saponins, anthraquinone glycosides and volatile oils using color reaction method. The results of phytochemical screening revealed the presence of alkaloids and volatile oils, but saponins and anthraquinone glycosides were found to be absent as shown in Table 1.

According determination chlorophyll and carotenoid contents were found that ethanolic *Gynura* extract (EGE) contained the contents of chlorophyll-a, b and carotenoids were 365.20±0.049, 132.40±0.029 and 53.20±0.034 μg g^{-1} dry weight, respectively.

The content of chlorophyll a and b in EGE has a constant ratio between chlorophyll a and chlorophyll b of approximately 3:1, whereas, the ratio between chlorophyll and carotenoid was approximately 9:1 as showed in Table 2.

The results of antibacterial activity of the extract at 80 mg mL^{-1} using agar well diffusion method. It was found that both aqueous and ethanol extracts of *G. procumbens* leaves have not inhibition zone as showed in Table 3.

The present study conclude that ethanolic leaves extract of *Gynura procumbens* extract is good natural source of bioactive compounds include chlorophylls, carotenoids, alkaloids and volatile oils. It may have beneficial health effects for consumption and could be further studied for uses in food industry, health product, pharmaceutical and medicinal applications.

Table 1: Phytochemical compositions of the ethanolic *Gynura procumbens* extract

Phytocompounds	Methods	Results	References
Alkaloids	Dragendorff's test	++++	Modified from De *et al.* (2010) and Ayoola *et al.* (2008)
	Hager's test	+++	
	Kraut's test	++++	
	Mayer's test	-	
	Valser's test	+	
Saponins	Frothing test	-	
Anthraquinone glycosides	Bomtrager's test	-	
Volatile oils	TLC test	++	

+: Positive results, -: Negative results

Table 2: Chlorophyll and carotenoid analysis of *G. procumbens* leaves extract

Phytochemical compounds	Average (A)	Contents (μg g^{-1} DW)	Ratio Chl a:b	Ratio Chl:Car
Chlorophyll a	0.794	365.20±0.049		
Chlorophyll b	0.453	132.40±0.029	2.76:1	9.35:1
Carotenoid	0.657	53.20±0.034		

Chl: Chlorophyll, Car: Carotenoid, A: Absorbance at 665.2, 652.4 and 470 nm of chl a, chl b and car, respectively, Mean±standard deviation of three experiments are presented

Table 3: Antibacterial effect of ethanolic and aqueous *G. procumbens* extracts

Test organism	Zone of inhibition (mm) AGE	EGE
Staphylococcus aureus	nz	nz
Salmonella typhi	nz	nz
Bacillus cereus	nz	nz
Pseudomonas aeruginosa	nz	nz
Escherichia coli	nz	nz

All assays were determined in triplicates, nz: No inhibition zone AGE: Aqueous *Gynura* extract; EGE: Ethanolic *Gynura* extract

DISCUSSION

Medicinal plants constitute an effective source of both traditional and modern medicines, herbal medicine has been shown to have genuine utility and about 80% of rural population depends on it as primary health care. Over the years, the World Health Organization (WHO) advocated that countries should interact with traditional medicine with a view to identifying and exploiting aspects that provide safe and effective remedies for ailments of both microbial and non-microbial origins (Akinyemi *et al.*, 2005). According to World Health Organization traditional medicines are relied upon by 65-80% of the World's population for their primary health care needs. Moreover, emergence of multiple drug resistant strains of microorganisms due to indiscriminate use of antibiotics to treat infectious diseases has generated a renewed interest in herbal medicine. The beneficial health effects of many plants, used for centuries as seasoning agents in food and beverages, have been claimed for preventing food deterioration (Kaur and Arora, 2009).

The present study extracted the medicinal plant *Gynura procumbens* leaves in ethanol and screened the phytochemical compositions which revealed the presence of alkaloids and volatile oils. These phytoconstituents may be responsible for various activities. These metabolites have been shown to be responsible for various therapeutic activities of medicinal plants (Tijjani *et al*., 2009). Phytochemical compounds can be used as a first line of therapeutic defense against cancer before chemotherapy and radiation treatment. Phytoconstituents in fruits and vegetables may reduce the risk of cancer, possibly due to dietary fibers, polyphenol antioxidants, antidiabetic, antimicrobial, anti-inflammatory and pain-relieving properties (Nwaogu *et al*., 2007; Agarwal *et al*., 2001; Ahmad and Beg, 2001; Vedavanam *et al*., 1999). Alkaloid is a mixed group or compound mostly contains nitrogen-bearing molecules (NH_2) that make them to be particularly pharmacologically active. There are different types of alkaloids, the principal among which are tropane alkaloids, sanguinarine, quinine alkaloid, berberine, reserpine, atropine etc. found in some plant (Kishore *et al*., 2009). Each form of alkaloids serves specific function in the body system. It is used in the traditional medicine for treating diarrhea, anti-tubercular, anti-inflammatory, antinociceptive and anti-pyretic properties (Kishore *et al*., 2009; Barbosa-Filho *et al*., 2006; Kupeli *et al*., 2002; Ivanovska and Philipov, 1996). One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms. These activities have been widely studied for their potential use in the elimination and reduction of human cancer cell lines (Nobori *et al*., 1994). Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects on humans and this has led to the development of powerful pain killer medications (Kam and Liew, 2002). Volatile oils are important plant constituents and many of them are strong antiseptic. Some volatile oils contain sesquiterpenes have anti-inflammatory, anti-arthritic, Antiviral, antioxidant and antimicrobial effects (Singh *et al*., 2007; Allahverdiyev *et al*., 2004). Findings from this study may be positive or negative results in some cases. However these suggested that *Gynura* extract containing phytocompounds which may be used as preliminary information and could be useful for further investigation.

Here, we measure the content of chlorophyll-carotenoid. Chlorophyll is the source of green color of fruits and vegetables. The extracts of green plants contain chlorophylls a and b as major pigments; with occur in the approximate ratio of 3:1 (Hojnik *et al*., 2007). It agrees well with our study. Chlorophylls are gaining importance increase in the human diet, not only as food colorants but also as healthy food ingredients (Fernandes *et al*., 2007). Some experimental data suggested also that chlorophyll may have some antioxidant and anticarcinogenic potential, it may help protect some toxins and it may ameliorate some drug side effect (Guil-Guerrero *et al*., 2003). Chlorophyll and its various derivatives have a long established history of use in traditional medicine and for therapeutic purposes (Guil-Guerrero *et al*., 2003) and antioxidant (Ferruzzi *et al*., 2002) properties. Photo-inhibition and oxidation can occur when plants are exposed to stress in the photosynthetic electron transport system which is the major source of Reactive Oxygen Species (ROS) in plant tissues. In the early study, we found that ROS may be scavenged effectively by the antioxidant system of the *G. procumbens* leaves. This indicated that the ratio between chlorophyll and carotenoid found in *G. procumbens* was higher than those in other green plants (Ferruzzi and Blakeslee, 2007). These results suggested that the Ethanolic *Gynura* Extract (EGE) may have health benefits.

However, the *G. procumbens* extracts showed negative effects on antibacterial activity in all microorganisms tested. This investigation indicated that the *Gynura* extract did not show antibacterial activity which similar to the recent study of Nazmul *et al*. (2011) which suggested that the methanolic *G. procumbens* extract has not show antifungal activity. However, negative results do not mean absence of bioactive constituents nor is that the plant inactive. Crude plant extracts are generally a mixture of active and non active compounds. Same observations have been reported earlier by various authors (Nazmul *et al*., 2011; Parekh and Chanda, 2007). Active compound (s) may be present in insufficient quantities in the crude extracts to show activity with the dose levels employed. Lack of activity can thus only be proven by using large doses. With no antibacterial activity, extracts may be active against other bacterial species which are not tested.

Our present study had shown that the *Gynura procumbens* leaves extract contain medicinally useful phytochemicals such as, alkaloids and volatile oils and it is a good of chlorophylls and carotenoids source. These substances are could be extracted food industry or health products as medicinal food, pharmaceutical exploits and researches in biology, biotechnology and general medicine. Further work may be isolation and characterize the bioactive compounds from this plant to evaluate their biological activities.

ACKNOWLEDGMENTS

Financial support from the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Commission on

Higher Education, Ministry of Education is gratefully acknowledged. We are also grateful to thank the Division of Research Facilitation and Dissemination, Mahasarakham University for Financial support and thank the Faculty of Medicine, Mahasarakham University for Research Grant No.54/01/001.

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