ANTIMICROBIAL ACTIVITY, ANTIOXIDANT CAPACITY AND CHEMICAL INVESTIGATION OF *EUPHORBIA MILII* var.splendens FLOWER

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Abstract

Myanmar medicinal plant (Kiss-me-quick red flowers) which is used for the treatment of diarrhea in Myanmar was selected to study in order to find the scientific basis for such use. The main aim of the present research was to evaluate the biological properties of *Euphorbia milii* var.splendens red flower. Phytochemical screening indicated that alkaloids, carbohydrates, flavonoids, glycosides, phenolic compounds, tannins, saponins, steroids, and terpenoids were observed. Determination of elemental analysis by atomic absorption spectrophotometry, it was observed that Ca, K, Fe, Zn, Mg and Na were present as essential trace elements in red flower petals. The antimicrobial activity of the red flowers was investigated by using agar well diffusion method. All the extracts possessed antimicrobial activity against all tested microorganisms (15 mm - 40 mm). *In-vitro* antioxidant capacity of watery and 70 % ethanol extract was determined by DPPH assay method. The antioxidant activity of the ethanol extract showed higher potency than that of the watery extract. The cyanidin was isolated from red flower petals by paper chromatographic method. The isolated compound was confirmed by spectroscopic method. Therefore, cyanidin containing Kiss-me-quick red flowers may be used as natural antioxidant and antimicrobial agent.

Key words: antioxidant, antimicrobial, cyanidin

INTRODUCTION

Plants have a significant role in maintaining human health and improving the quality of human life. The world health organization (WHO) estimated that 80 % of the people rely on traditional medicine. The medicinal plant, *E. milii* var.splendens flower, belonging to the family, Euphorbiaceae, is commonly known as "Kiss-me-quick". *E. milii* is a native shrub of the Island of Madagascar. It is a cultivated plant and it could be found in many parts of the world such as USA, Brazil, China, Myanmar and other tropical regions. In Chinese folk medicine, euphorbia compounds were used in cancer treatment (Lee, 1982). Scientific investigation on antimicrobial activity and antioxidant capacity of Kiss-me-quick red flowers are still lacking. In the present research work, Kiss-me-quick red flowers were selected for investigation of phytoconstituents, elemental analysis, antimicrobial activity, antioxidant activity and some chemical constituents.

MATERIALS AND METHODS

Plant material

Red flowers of *E. milii* var.splendens (Kiss-me-quick) were collected from local market, Yangon Region. The collected red flower was confirmed as *Euphorbia milii* var.splendens at Botany Department, Dagon University.

Determination of elements

For determination by AAS, about 0.1 g of ash sample was accurately weighed and dissolved in 2 cm^3 of concentrated hydrochloric acid. The resulting solution of ash sample was evaporated to dryness and dissolved in 6 cm^3 of 25 % HCl solution (volume by volume) followed by centrifugation. The centrifuged solution was decanted and the clear solution was made up to 100 cm^3 with deionized water. The resultant solution was ready for analysis of mineral elements by AAS.

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Preparation of crude extracts

The fresh red petals sample (20 g) were percolated in pet-ether (200 mL) with occasional shaking for one month and filtered. The filtrate was concentrated on a water bath. Pet-ether crude extract was obtained. Similarly, the crude extracts of 95 % EtOH and EtOAc were obtained. In the preparation of watery extract, 20 g of dried powdered sample was soaked in distilled water (200 mL) in the conical flask. This flask was boiled for about 30 mins on a hot plate, cooled and filtered with filter paper. This process was carried out for three times. The combined filtrates were evaporated on a hot plate and dryness in the oven at 100 °C to get watery extract.

Preparation of anthocyanidin

The fresh red petals (20 g) was dissolved in 2M HCl acid solution for 45minutes on a water bath and filtered. The filtrate was partitioned with ethyl acetate. Then, the ethyl acetate insoluble layer was partitioned with amyl alcohol. When the amyl alcohol layer was evaporated to dryness, anthocyanidins crude extract was obtained (Harborne, 1984).

Screening of antimicrobial activity

Antimicrobial activity of the four crude extracts was determined by agar well diffusion method. Four small holes of 10 mm diameter each were cut out in the inoculated agar to place samples to be tested. The volume of each sample placed in each hole was 0.1 cm^3. The petridish was then incubated at 37 ºC for 48 hours, and the diameters of clear inhibition zones around the holes, if appeared, were measured (Finegold,1982).

Determination of antioxidant activity

DPPH radical scavenging activity of 70 % ethanol and watery extracts of *E. milii* var.splendens flowers was determined by UV-visible spectrophotometer. The control solution was prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of the ethanol in the brown bottle. The sample solution was also prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of tested sample solution. These bottles were incubated at room temperature and were shaken on shaker for 30 minutes. After 30 minutes, these solutions were measured at 517 nm and the percentage of radical scavenging activity (% RSA) was calculated. The antioxidant power (IC_{50}) (50 % inhibition concentration) values were calculated by linear regressive excel program (Marinova *et al*., 2011).

Isolation of compounds

The chromatographic tank was saturated with solvent vapor for one hour after formic acid solvent (Conc. HCl: HCOOH: H_2O, 2:5:3) was added. The anthocyanidin residue was dissolved in methanol. Then the extract was applied in a straight line on the Whatman No.3 paper and allowed to dry. The paper was developed with solvent. After the paper has dried, the pink fraction was eluted with 1 % methanolic hydrochloric acid solution and the extract was evaporated under reduced pressure. The residue was used for identification.

RESULTS AND DISCUSSION

In the present work, the red flower sample was tested on six strains of bacteria which include *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli*. The measurable zone diameter, including the agar well diameter, shows the degree of antimicrobial activity. It was found that all the extracts of red flowers showed antimicrobial activity against all tested microorganisms (Figure 2 & Table 2). According to the result of antioxidant activity, the IC_{50} values of watery

and ethanol extracts were 2.24 µg/mL and 2.18 µg/mL, respectively (Table 3). The lower the IC_{50} value, the higher the antioxidant activity. So, the antioxidant activity of ethanol extract possessed higher potency than that of watery extract. Crude anthocyanidin extract was separated by ascending preparative paper chromatography using formic acid solvent (Figure 1). The wavelength of maximum absorption was found at 538 nm for pink fraction, cyanidin (Figure 3). The isolated compound was also compared with reported UV spectral data.

Table 1. Elemental Contents in Red Flowers of *E. milii*

Sr.No	Elements	Percentage (%)
1.	Ca	0.089
2.	Zn	0.041
3.	Mg	0.054
4.	Fe	0.046
5.	K	0.039
6.	Na	0.013

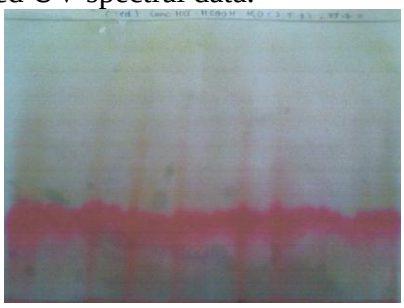

Figure 1. Paper chromatogram of red flowers of *E. milii*

Table 2. Antimicrobial Activity of Red Flowers of *E. milii* (Kiss-me-quick)

No.	Tested organisms	Inhibition zone diameters (mm)			
		PE	EtOAc	EtOH	Anthocyanidin
1.	*Bacillus subtilis*	35	28	22	15
2.	*Staphylococcus aureus*	38	28	22	28
3.	*Pseudomonas aeruginosa*	40	30	25	30
4.	*Bacillus pumilus*	35	38	20	25
5.	*Candida albicans*	35	30	22	25
6.	*Escherichia coli*	36	28	20	26

Table 3. Oxidative Inhibition % and IC_{50} Values of Watery and EtOH Extracts of *E. milii*

Extracts	% Oxidative inhibition (Mean±SD) (µg/mL)					IC_{50} (µg/mL)
	0.625	1.25	2.5	5	10	
H_2O	12.99±0.51	27.68±0.33	55.93±0.33	73.45±1.47	90.96±0.98	2.24
EtOH	17.51±1.13	31.07±0.78	56.49±1.25	81.36±0.78	93.79±1.31	2.18
Ascorbic Acid	24.29±0.50	63.28±1.11	89.27±0.12	89.27±1.37	96.05±0.61	1.03

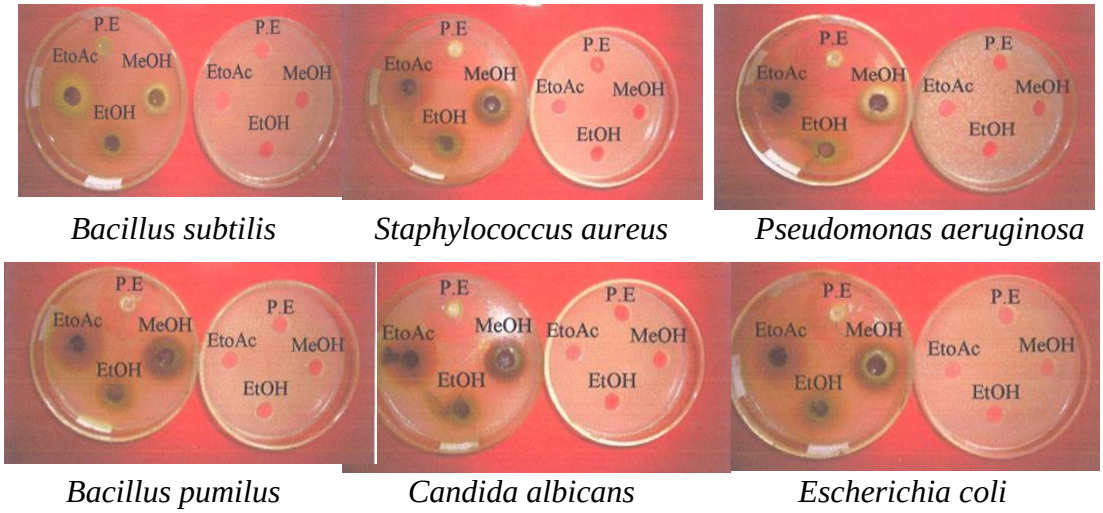

Figure 2. Antimicrobial screening of red flowers of *E. milii* (Kiss-me-quick)

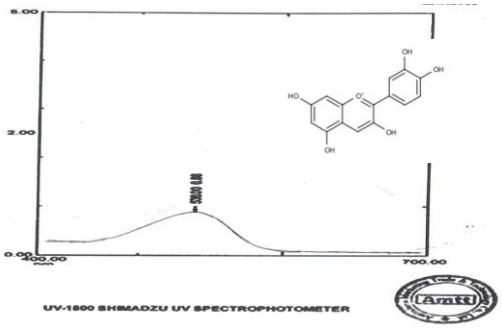

Figure 3. UV spectrum of isolated cyanidin from red flowers of *E. milii* (Kiss-me-quick)

CONCLUSION

From the present research work on "Antimicrobial activity, antioxidant capacity and chemical investigation of *Euphorbia milii* var.splendens flower", the following conclusions can be drawn.

The phytochemical investigation of the selected red flower revealed the presence of alkaloids, flavonoids, glycosides, phenolic compounds, saponins, steroids, tannins and terpenoids but starch was not detected. Elemental analysis of plant sample by AAS method, Ca, K, Mg, Fe, Na and Zn were present as essential trace elements in selected red flowers. The crude extracts such as (PE, 95% EtOH, EtOAc and anthocyanidin) from red flowers showed antimicrobial activity against six microorganisms tested (15 mm-40 mm). The smaller IC_{50} value of ethanol extract showed more potent antioxidant capacity than watery extract. By paper chromatographic separation technique, cyanidin was isolated and confirmed by spectroscopic method and also by comparing with its reported data. In conclusion, ethanol extract of the red flowers possessed significantly antimicrobial activity and antioxidant activity. From the experimental results, the extracts of *E. milii* (Kiss-me-quick) red flowers are suitable for economic production of antimicrobial agent for oral preparations.

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