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DPPH free radical scavenging activity of crude and fractionated extract and stability of *Ruellia tuberosa*' s fractionated extract in cream product

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ABSTRACT

The study of antioxidant activity by DPPH radical scavenging method of crude extract from *Ruellia tuberosa*'s leaves, stems and roots were extracted with two solvents as ethanol and acetone to afford six segmental extracts. The ethanol solvent showed higher efficiency crude extraction than acetone solvent. When, all crude extracts were investigated by DPPH free radical scavenging assay. The antioxidant activity of leaves stems and roots were extracted by ethanol as 79.42%, 79.52% and 54.80%, respectively and acetone as 76.52%, 88.25% and 77.74%, respectively. The DPPH activity of stem extracted with acetone (AS) showed highest activity. Then, acetone extract of stem was fractionated with column chromatography technique by using gradient system of hexane:ethyl acetate to be obtained 11 fractions. All fractions were further to study of DPPH free radical scavenging activity. The fractioned 7 of stems' acetone extract (AS-F7) obtained sticky yellow-brow (48.5 mg), showed percentage of activity at 85.31%. The followed up stability of AS-F7 at 1 and 2% (wt/wt) mixed in skin cream base for 4 weeks ago at room temperature compared with cream base (control). The study result, AS-F7 from *R. tuberosa* showed stability of antioxidant activity in cream base, which lost percentage of antioxidant activity less than cream base without added extract. It can be applied to active ingredient in skin cream products. The customers were satisfaction survey of skin cream products questionnaire by using purposive sampling. The physical property result showed texture, touch, color, senses, viscosity, feeling on skin and overall product satisfaction were 4.69, 4.62, 4.69, 4.62, 4.23, 4.23 and 4.69 respectively.

Keywords: *Ruellia tuberosa*, antioxidant activity, DPPH free radical scavenging assay, skin cream product

1. INTRODUCTION

Ruellia tuberosa L., belong to the family Acanthaceae Thai name: Toi-ting, is a perennial herb and widely distributed in tropical area of India, Taiwan and Thailand. It has different names such as fever root, cracker plant and minnie root. This plant can be easily found in open waste or moisture place. *R. tuberosa* has been great importance due to their nutritive value [1] and externally used in Thai tradition medicine as an antiseptic, anti-inflammatory, anti-ulcer [2]. Previous phytochemical studies, of this plant revealed the presence of antioxidant compounds from *R. tuberosa* were ascorbic acid, lycopene, carotenoid, tocopherol [1]. Phenylethanoid glycoside showed relative scavenging activity in same range of ascorbic acid [2]. In 2006, Chen, F.A. reported antioxidant activities of the different fractions from stem tested decreased in the order of ethyl acetate > chloroform > methanol > water > hexane fraction [3]. The flavonoid which isolated from ethyl acetate extract of dried aerial parts showed cytotoxicity against KB cell and HepG2 [4] and used for ulcer protective activity in male wistar rats [5]. *In vitro* antioxidant activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *R. tuberosa* was evaluated by studying 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, hydroxyl radical scavenging activity, superoxide radical scavenging activity, ABTS radical cation scavenging activity and reducing power using standard procedure. Among the solvents tested, methanol and ethanol extracts of tuber of *R. tuberosa* showed potent *in vitro* antioxidant activities. The results clearly indicated extracts of tuber of *R. tuberosa* is effective in scavenging free radicals and has the potential to be a powerful antioxidant[6].

The effect of free radicals wrathful by various environmental chemicals as well as endogenous metabolism are involved in a number of diseases like tumors, inflammation, gastric mucosal injury, atherosclerosis, shock, diabetes, infertility, and ischemia due to the oxidative damage to DNA, lipids, and proteins and which can result in failure of cellular functions. So, human try to useful plant material for free radical scavenging applied to dietary supplement and cosmetic product. The present study attempted to estimate the antioxidant activity of *R. tuberosa* was several method by 2,2-diphenyl-1-picrylhydrazyl (DPPH). In this work, we study antioxidant activity of crude extract of leaves, stems and roots from *R. tuberosa* and fractionated extract by DPPH free radical scavenging assay. The active fraction extraction was applied to ingredient skin cream product. All product skin creams were studied stability and customer satisfaction survey of skin cream products questionnaire by purposive sampling.

2. MATERIALS AND METHODS

Plant material

Ruellia tuberosa was collected from Ramkhamhaeng University of Thailand was used as plant material.

Extraction and Column Chromatography

Chemical

Acetone, Ethanol, Ethyl acetate, Hexane (AR grade: RCI Labscan), 1,1-

diphenyl-2-picrylhydrazyl;DPPH (Sigma Aldrich)

The fresh plant part was washed and picked impurity out of them. The plant material was separated for three parts as leaves, stems and roots. Each part of material was crushed with blender and subjected to extraction by macerating. The extract was taken by soaking the fresh of leaves, stems and roots in two difference solvents (ratio plant: solvent; 1:2) as acetone or ethanol at room temperature for 7 days (3 times). Then, the samples were filtrated through Whatman filter paper No.2. The solvents of the respective combined extracts were evaporated under reduces pressure, using a rotary vacuum evaporator (RotavaporR-210, Heating Bath B-491, Vacuum Pump V-700, CTL 911) at 40 °C to afford 6 crude extracts as ethanolic extract of leaves (EL), stems (ES), roots (ER) and acetone extract of leaves (AL), stems (AS), roots (AR). All crude extracts were determined antioxidant activity by DPPH free radical scavenging assay.

Formulation and Development of Cream Base

Cremophor A-6, Cremophor A-25, Finsolv TN, White oil 2076, G.M.S, Wax-C, Propylene glycol, Unigerm G2 and Alpha bisabol. The oil phase prepare by heating Cremophor A-6, Cremophor A-25, Finsolv TN, White oil 2076, G.M.S, Wax-C to 75 ±1 °C. At the same time, the aqueous phase included water and propylene glycol was heated to the same temperature. The aqueous phase was subsequently added to oil phase drop by drop with continued stirring at 2,500 rpm by a mechanical mixer for 15 min. During this stirring time, Unigerm G2, Alpha bisabol and the most active antioxidant of fractionated (1, 2% wt/wt) from *R. tuberosa* were homogenized with cream base. The same method was used to formulate the cream base without the addition active fractionated extract of *R. tuberosa*. The viscosity of cream base was measured by Viscometer (LD VD-II+Pro) in centipoise (cPs).

Determination of Antioxidant Capacity

Antioxidant activity (DPPH free radical scavenging assay) crude extracts of ethanol and acetone were determined by using the DPPH method. Briefly, crude extract solution (1,000 ppm) mixed with DPPH solution (0.2 mM) ratio 3:1 and incubated in the dark at room temperature for 30 min. The absorption of sample was measured at 517 nm (UV-VIS SPECTROPHOTO-METER series V-650 spectrophoto-meter). Decreasing of the DPPH solution absorbance indicates increase of the DPPH radical scavenging activity. The highest antioxidant of crude extract was selected to fractionate by using column chromatography with gradient solvent system (hexane:ethyl acetate; 100:0 to 0:100). All fractionates were test antioxidant activity. The highest antioxidant activity was selected to ingredient in cream product at 1% and 2% wt/wt. After that, the products with/without fractionated extract were determination activity by DPPH method for 4 weeks ago at room temperature. The percentage of antioxidant activity by DPPH assay was using the following equation:

$$Activity(\%) = \frac{(Abs_{DPPH} - Abs_{sample})}{Abs_{DPPH}} \times 100$$

Where Abs_{DPPH} =Absorbance of DPPH
Abs_{sample}=Absorbance of sample

Customer Satisfaction Survey

The customer satisfaction survey of skin cream product was purposive sampling. The titles of testing were physical property as texture, touch, color, senses, viscosity, feeling on skin and overall product satisfaction.

3. RESULTS AND DISCUSSION

The fresh leaves, stems and roots from *R. tuberosa* were extracted with ethanol and acetone solvent to afford six segmental extracts as ethanol leaves (EL), ethanol stems (ES), ethanol roots (ER), acetone leaves (AL), acetone stems (AS) and acetone root (AR). The fresh plants weight (wt.), weight of crude extract and percentage yield of crude extract showed in Table 1.

Table 1. Weight of fresh plant, extraction and %yield of *R. tuberosa*'s leaves, stems and roots.

Part of fresh plants (solvent)	Plants wt. (g)	Extract wt. (g)	% yield of extract
Leaves (ethanol)	1020.00	25.32	2.48
Stems (ethanol)	390.00	14.00	3.59
Roots (ethanol)	1000.00	78.76	7.88
Leaves (acetone)	250.00	3.87	1.55
Stems (acetone)	300.00	6.28	2.09
Roots (acetone)	200.00	4.44	2.22

The efficiency of ethanol solvent could be extract fresh leaves, stems and roots gave higher %yield crude extracts than acetone solvent.

Determination of Antioxidant Capacity

All crude extracts (EL, ES, ER, AL, AS, AR) were investigated by DPPH free radical scavenging assay indicated in Table 2, 3 and Figure 1.

Table 2. The percentage antioxidant activity of ethanol extract at 1,000 ppm.

Sample No.	Absorbance value at 517 nm		
	leaves (EL)	stems (ES)	roots (ER)
1	0.4637	0.4866	1.0414
2	0.4927	0.4795	1.0199
3	0.4690	0.4403	1.0426
average	0.4751	0.4688	1.0346
S.D.	0.0017	0.0032	0.0022
C.V.	0.3597	0.7000	0.2122
% Activity	79.2437	79.5203	54.8017

The antioxidant activity of crude extract showed high to low activity as AS (88.25%), ES (79.52%), EL (79.24%), AR (77.74%), AL (76.25%), ER (54.80%). The AS crude extract showed higher activity, then choose for fractionated by column chromatography.

Table 3. The percentage antioxidant activity of acetone extract at 1,000 ppm.

Sample No.	Absorbance value at 517 nm		
	Leaves (AL)	Stems (AS)	Roots (AR)
1	0.5478	0.2695	0.5204
2	0.5304	0.2690	0.5105
3	0.5528	0.3685	0.5105
average	0.5437	0.2690	0.5097
S.D.	0.0020	0.0020	0.0026
C.V.	0.3688	0.7587	0.5152
% Activity	76.2497	88.2487	77.7350

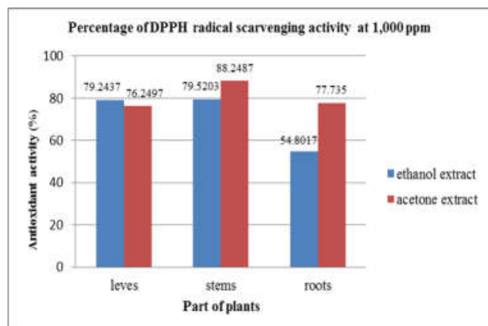

Figure 1. Antioxidant activity of crude extracts

Colum Chromatography

The acetone extract of stem (AS) crude extract was separated by using column chromatography technique with gradient solvent (hexane:ethyl acetate, 100:0 to 0:100) system detected and collected of fraction by thin layer chromatography to obtain 11 fractions. The AS-F1 to F11 fractions were weight as 49.4, 7.5, 244.8, 76.1, 120.4, 75.0, 48.5, 2.8, 3.5, 29.2 and 7.7 mg, respectively. Then all fractions were prepared 1,000 ppm concentration and tested antioxidant activity show in Table 4.

Table 4. The percentage antioxidant activity of AS-F1 to AS-F11 at 1,000 ppm

ID Code	Wt. (mg)	Absorbance value at 517 nm				S.D.	C.V.	% Activity
		1	2	3	average			
AS-F1	49.4	0.8058	0.806	0.806	0.8059	0.0001	0.0129	60.7495
AS-F2	7.5	0.3302	0.3304	0.3308	0.3304	0.0003	0.0876	83.9041
AS-F3	244.8	0.5329	0.5329	0.5328	0.5328	0.0001	0.0143	74.0439
AS-F4	76.1	0.7512	0.7511	0.7511	0.7511	0.0001	0.0100	63.4092
AS-F5	120.4	0.6606	0.6609	0.6612	0.6609	0.0003	0.0429	67.8034
AS-F6	75.0	0.7095	0.7093	0.7095	0.7094	0.0001	0.0143	65.4406
AS-F7	48.5	0.3019	0.3015	0.3013	0.3016	0.0003	0.1048	85.3072
AS-F8	2.8	0.2583	0.2582	0.2584	0.2583	0.0001	0.0333	87.4166
AS-F9	3.5	0.4947	0.4952	0.4954	0.4951	0.0004	0.0759	75.8805
AS-F10	29.2	0.3161	0.3166	0.317	0.3165	0.0005	0.1426	84.5813
AS-F11	7.7	0.1028	0.1026	0.1026	0.1027	0.0001	0.1425	94.9968
DPPH (0.2 mM)	664.9	2.0532	2.0527	2.0523	2.0527	0.0005	0.0224	-

Table 5. The percentage antioxidant activity of AS-F7 in base cream at 1,000 ppm

Sample	Absorbance value at 517 nm						
	1	2	3	average	S.D.	C.V.	% Activity
1st week							
0 %	0.7044	0.7051	0.7053	0.7049	0.0005	0.0650	47.8291
1 %	0.6910	0.6904	0.6892	0.6902	0.0009	0.1330	48.9195
2 %	0.6720	0.6711	0.6706	0.6713	0.0007	0.1024	50.3232
DPPH (0.2mM)	1.3510	1.3541	1.3486	1.3512	0.0027	0.2029	-
4th week							
0 %	1.4162	1.4159	1.4153	1.4158	0.0005	0.0321	43.2909
1 %	1.3837	1.3829	1.3821	1.3829	0.0008	0.0593	44.6087
2 %	1.3112	1.3107	1.3101	1.3107	0.0006	0.0442	47.5019
DPPH (0.2 mM)	2.4966	2.4973	2.4959	2.4966	0.0007	0.0280	-

The five fractions, AS-F2, AS-F7, AS-F8 AS-F10 and AS-F11, showed higher activity (>80%). Whereas, AS-F7 has more weight than another faction in this group so, AS-F7 faction (sticky oil yellow-brow; 48.5 mg) was selected to mix with cream base and to study stability of antioxidant activity in cream base (Table 4).

Formulation and Development of Cream Base

The physical property of cream base was white color emulsion. The viscosity was measured 71,085 cP by viscometer (LD VD-II+Pro) needle no 4(64), speed 6 rpm for 2 min at room temperature 24 °C. The physical property of skin cream product has ingredient AS-F7 at 1 and 2% (wt/wt) were yellowish but 2%wt/wt has more color 1% (wt/wt). Determination of skin cream product's antioxidant stability: the followed up stability of AS-F7 at 1 and 2% (wt/wt) mixed in skin cream base for 4 weeks ago at room temperature compared with cream base (control). After that, check the pattern of UV absorption of samples in first time exhibited in figure 2.

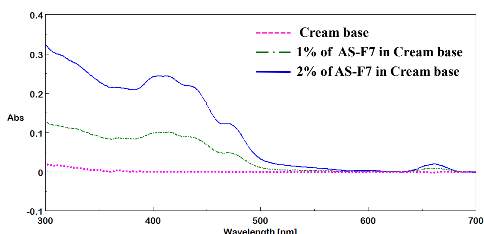

Figure 2. UV spectrum of cream base and cream base mixing with AS-F7 extract at 1, 2% wt/wt.

The cream base lost percentage of antioxidant activity as 4.54 whereas cream base was mixed with AS-F7 at 1 and 2 %wt/wt have lost percentage of antioxidant activity at 4.31 and 2.82, respectively, after followed up stability for 4 weeks ago at room temperature. (Table 5).

Customer Satisfaction Survey

The customer satisfaction survey of skin cream products by purposive sampling found that physical property as texture, touch, color, senses, viscosity, feeling on skin and overall product satisfaction were 4.69, 4.62, 4.69, 4.62, 4.23, 4.23 and 4.69 respectively.

4. CONCLUSION

In this research the ethanol solvent had efficiency extraction than acetone, and all crude extracts were tested the antioxidant activity by DPPH assay. The stem crude acetone extract has been showed higher activity than another them. Then, stem crude acetone extract was separated by column chromatography technique to afford 11 fractions. The five (AS-F2, AS-F7, AS-F8 AS-F10 and AS-F11) from eleven fractions showed antioxidant activity more than 80%. The AS-F7 obtained sticky yellow-brow showed percentage of activity at 85.31% was selected mixing in cream base at 1, 2% wt/wt. The stability of antioxidant of AS-F7 was studied for 4 week. It found that 2%wt/wt has low lost percentage of antioxidant than 1%wt/wt and cream base. The skin cream product has *Ruellia tuberosa* extract in ingredient, was tested by customer satisfaction survey questionnaire of skin cream products by purposive sampling average all questions at level 4.54.

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REFERENCES

1. Manikandan, A., & Victor Arokia Doss, D. Evaluation of biochemical contents, nutritional value, trace elements, SDS-PAGE and HTPLC profiling in the leaves of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.). ***J. Chem. Pharm. Res***. 2, 3 (2010), 295-303.
2. Phakeovilay, C., Disadee, W., Sahakitpichan, P., Sitthimonchai, S., Kittakoop, P., Ruchirawat, S., & Kanchanapoom, T. Phenylethanoid and flavone glycosides from *Ruellia tuberosa* L. ***J. Nat. Med***. 67 (2013), 228-233.
3. Chen, F.A., Wu, A.B., Shieh, P., Kuo, D.H., & Hsieh, C.Y. Evaluation of the antioxidant activity of *Ruellia tuberosa*. ***Food Chemistry***. 94 (2006), 14-18.
4. Lin, C.F., Huang, Y.L., Cheng, L.Y., Sheu, S.J., & Chen, C.C. Bioactive flavonoids from *Ruellia tuberosa*. ***J. Chin. Med***. 17, 3 (2006), 103-109.
5. Praveen SriKumar, P., & Pardhasaradhi, P. Preliminary phytochemical investigation and anti-ulcer activity of aerial parts of *Ruellia tuberosa* L. (Acanthaceae) in male Wistar rats. ***Int. J. Pharm. Biomed. Res***.4, 3 (2013), 145-148.
6. Rajendrakumar, N., Vasantha, K., Murugan, M., & Mohan V. R. Antioxidant activity of tuber of *Ruellia tuberosa* L. (Acanthaceae). ***Int. J. of Pharm. and Phytochem. Res***. 6, 1 (2014), 97-103.

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