International Journal of Pharmaceutical Science Invention
ISSN (Online): 2319 – 6718, ISSN (Print): 2319 – 670X
www.ijpsi.org Volume 6 Issue 6 ǁ June 2017 ǁ PP. 15-21

Optimization of the treatment and extraction procedures for Cetellaasiatica L. Urban.

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Abstract: *The study is to research and apply enzyme Viscozyme supported for increase the possibility of constituents from Centellaasiatica (CA) that has biological effectiveness. The results show that the Centellaasiatica extract (CAE) yield is higher twice when comparing with the classical extractmethod (only using ethanol solvent). The detailed results: Surveying pre-treatment conditions of Viscozyme reached to the highest effectiveness at the concentration 2%, at 45^{0}C, in 90 minutes with Centella/ enzyme: 1/8. After CA treated with by enzyme will be extracted twice with ethanol 80%, in 60 minutes and at 40^{0}C with Centella/enzyme: 1/9 (w/v) whichreached 46,22%. The optimization conducted RSM-CCD Minitab 16 software with the results: CA treated withViscozyme 2,5%, at 43^{0}C. Then, extracted three times by ethanol 75% with mixture ratio ratio of solid to solvent (1/9 w/v), the optimized CAE yield is 49,21%, The method of optimization is used Minitab 16 that helped to reducethe extracted ethanol concentration(5%), temperature at 5^{0}C, also reducing the extracted time concentration (5,5 hours) when comparing with the old method. The extraction of asiaticoside from optimized CAE process (RMT) reached 37,91 mg/ml, higher 2,32 times when comparing with the classical extract method (RM1) only reached 16,31 mg/ml. The study has applied enzyme in the process which helped the time of the extracted process shorter, reducing temperature and the concentration of the extracted solvent, increasing the content of extracted asiaticoside.*

Keyword:*Extracted Centellaasiatica, Asiaticoside, Viscozyme L, RSM-CCD, Minitab 16.*

I. Introduction

Today, using medicines from natural origin has been trended to atraction. Many researches show that using natural medicines is less harmful effectiveness. That is the reason why a lot of natural medicines are studied more and more.CA (*Centellaasiatica*) contains saponin triterpenoid as: asiaticoside, madecassoside,... In that, asiaticoside is a factor effecting to connective tissue creativity, helping to create new skin to healing the wound quickly. It can be applied widely in medicine and cosmetics field. Besides, asiaticoside is used in leprosy treatment, tuberculosis treatment, slower the process of aging [6].

Moreover, Centella is also a kind of herb having a lot of good nutrition, vitamins, minerals, antioxidants using in therapy, improving in memory, slowing the process of aging, improving in circulatory system and treating skin diseases.... [2], [5]. Therefore, the extraction of centella's compounds is able to apply beverages and create the new products helping to improve human health, increasing the marketing value of CA to increase the profit for farmers.

II. Materials Andmethods

Centellaasiatica (L.) Urban has bought in Hiep Thanh village, Go Dau district, Tay Ninh province.

Experimental Equipments: Weighing moisture analyzer MX 50-AND (Japan), evaporator (Bibby Stuart - England), Vacuum pump (Woosung-Korea), Shaking Water Bath (Jeio tech – Korea), Drying oven (Labtech - Korea), HPLC (Waters 2690 - Germany).

Enzyme: Viscozyme L (Novozymes-USA).

2.2. Method

2.2.1. Processing materials

Centellaasiatica (CA) was dried at 400C, humidity under 10%. Then, it was grinded to power (2mm < d < 3mm), using for the process of *Centellaasiatica*extract (CAE) in study [6], [13].

2.2.2 Extraction method

Centella powder was immersed in Viscozyme solution in the concentration 1 - 3 %, 40- 60^{o}C, 60 - 180 minutes, centella/enzyme 1/7-1/11. Then, CAE obtained by extracting under the following conditions: ethanol concentration 20 – 90%, around 30 – 240 minutes, 30 – 70^{0}C, Centella/ethanol 1/7 -1/11 with the times of extraction 1 – 5 times. The speed of stir was stable in 400 rounds/ minute. Centella was filtered in whatman filtering device at the pressure 100psi to extrude the impurities, the extracted liquid from Centella input to evaporator at 45^{0}C at the pressure 760mmHg to collect Centella [7].

2.2.3. Determining the efficiency of collected dried *Centella asiatica* extract (TCVN 5533 - 1991)

Filtered liquid from centella was evapored and determined the efficiency of dried collection following:

$$\%Y=[(1-x2)*m2]/[(1-x1)*m1]*100$$

m1: dried weight in the first material (g)
m2: dried weight in the product (g)
x1: The humidity of the first material (g)
x2: The humidity of the finished material (g)
The experiments wereconducted 3 times, the data from surveying the optimum condition in the single factor has done and classified test by MSTATC software.

2.3. Otimized software

Optimizing the CAEconducted by the program Minitab 16.2.3.0. Experimental designs and data analysis were performed using Minitab 16 software (Minitab Inc-USA). ANOVA procedures were performed at confidence level of 0.95.

III. Results And Discussion

3.1. Surveying the single factor of extracted Centellaconditions by enzyme

3.1.1. The conditions for processing Centella by Viscozyme L

Viscozyme is complex compounds containing a lot of Carbohydrates (cellulase, arabanase, pectinase, pentosanase, xylanasse). Xylanase makes break hemicellulose, one of the main components of cell wall. Cellulase is a complex group of enzymeshad an effect on cellulose hydrolysis through the hydrolysis of the connection 1, 4-β-glucoside in cellulose.

Surveying to collect suitable temperatures by enzyme reached the highest value (45,21%) at 45^{o}C, the suitable information that the company provided (25-55^{o}C), temperature at 45^{o}C is the optimum point for enzyme Viscozyme impact.

Table 1: Surveying the influence of incubatedmaterial by enzyme to the *Centellaasiatica* extract

Concentration (%)	1	1,5	2	2,5	3
	28,71^{d}	30,89^{c}	33,17^{b}	33,24^{ab}	33,24^{a}
Centella/enzyme (w/v)	1/7	1/8	1/9	1/10	1/11
	32,57^{c}	34,20^{b}	34,29^{ab}	34,34^{a}	34,39^{a}
Temperature (^{o}C)	40	45	50	55	60
	39,88^{c}	41,81^{a}	41,75^{ab}	41,18^{b}	39,56^{d}
Time (minute)	60	90	120	150	180
	34,52^{c}	41,96^{a}	42,01^{a}	42,10^{a}	39,05^{b}

Surveying the effect of single factor to the efficiency of collected dried centella reached highest yield when processing by Viscozyme at the concentration of enzyme (w/v) 2,5%, 1/8(w/v) enzyme solution-to-meterial ratio, at 45^{0}C, in 90 minutes, the extract yield reached 41,96%.

The results of the table 1 is similar to the conclusion in the study of Leng *et al.* (2006), using material/enzyme (w/v) is 1/5 in processing sun flower seed to collect pure oil (collected efficiency 85,73%). Marcello Salvatore Lenucci *et al.* (2015) processing 1 liter liquid of tomatoes with the concentration 0,25% Cellulast/Viscozyme (v/v) 3/1 getting the collected efficiency of lycopene 44 - 67%.

3.1.2. Pre-treatment conditonsfor extracting CA processed by enzyme

To evaluate the CAE through enzyme process comparing withthe solvent extraction method. We got the following results:

Table 2: Surveying the effection of extracted Centella conditions by ethanol processed enzyme

Concentration (%)	20	40	60	80	90
	38,51^{c}	39,32^{b}	42,54^{ab}	42,74^{a}	42,96^{a}
Centella/enzyme (w/v)	1/7	1/8	1/9	1/10	1/11
	36,86^{c}	36,91^{c}	40,10^{b}	41,83^{a}	41,98^{a}
Time (minute)	30	60	120	180	240
	31,30^{c}	42,35^{a}	42,13^{ab}	42,05^{b}	42,00^{b}
Temperature (^{o}C)	30	40	50	60	70
	38,22^{c}	43,07^{a}	42,19^{b}	42,07^{b}	42,15^{b}
Times of extraction	1	2	3	4	5

(times)	45,18[b]	46,22[a]	46,34[a]	46,42[a]	46,49[a]

Hamid (2002), when extracting by ethanol 99,9% in 24 hours, the extract yield only reached 28,3%. Chippada (2011) extracting in 48 hours, extract yield about 13,37%. HoaiThuong Nguyen (2011) extracting by ethanol 96%, yield extract was about 1,75%. Pre-treatment with enzyme, and then*Centellaasiatica* (CE) extracted by ethanol help to cut down the time 20 hours in extraction, temperature in 40^0C and ethanol concentration that it was lower than the solvent extraction method. That had the great meaning because it reduced the impacts of solvent. Because the temperature and the time of extraction is shorter, it increases the bioactivity of CAE.

3.2.1. Screening design for the main factors affect in the extraction yield

The Optimized model of the elements has designed by Mini Tab software.

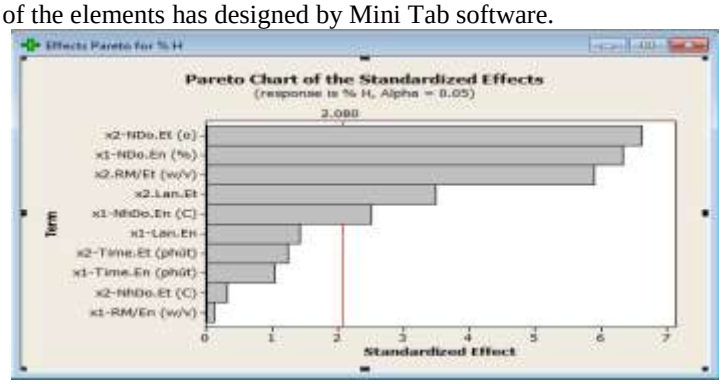

Fig 1. Evaluating the suitable level R-Sq (adj)

3.2.1. Screening the affected factors

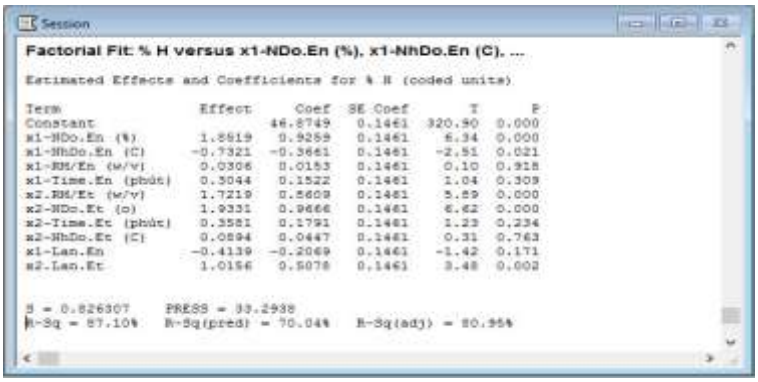

Fig 2. Factors in the Plackett–Burman Screening Design.

After screening, 5 main factors affected to extract yield: ethanol concentration, Centella/ethanol and the times of extraction by using ethanol is one of the main elements affecting the results of the affected elements correlated with the previous studies' results of Linh Tuyen Nguyen Thi et al. (2011) [14] assumed that ethanol concentration, ethanol/Centella and the times of extraction were 3 factors affected to collected dried efficiency [14]. From those affected factors in fig.1, we carried out CCD design aimed for finding point of design to help CAEreach to maximum yield when extracted at thatoptimized point.

3.2.2. Building the experiment schedule equation

Base on the results of the regression model (fig.3). Firstly, we saw the row (Lack-of-Fit) of the regression model level 2 (Full quadratic) had value p = 0.103 greater when comparing with meaning $\alpha = 0,05$ (Fig 4). This is the model adapted to data with reliability 95%.

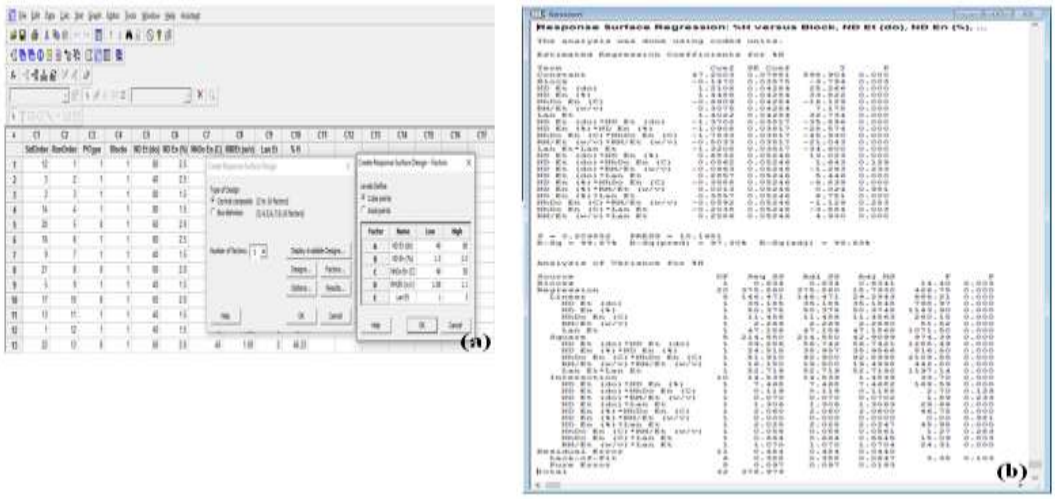

Fig 3. Design and analyze a response surface
(a). Central composite matrix design for eight runs; **(b).**The result of variance and regression of CCD experiment

Base on the results of the regression model (fig.3). Firstly, we saw the row (Lack-of-Fit) of the regression model level 2 (Full quadratic) had value p = 0.103 greater when comparing with meaning $\alpha = 0,05$ (Fig 3). This is the model adapted to data with reliability 95%.
By the analyzing results of ANOVA by Minitab software 16, we got the following regression:

Y=7,20+1,21X1+1,44X2-0,69X3+0,30X4+1,40X5-1,37X12-1,09X22-1,75X32-0,80X42-1,32X52+0,28X1X2+0,28X1X5-0,35X2X3+0,35X2X5-0,20X3X5+0,25X4X5

X1 – ethanol concentration, X2 – enzyme concentration, X3 – enyzme temperature, X4 - Centella/ethanol, X5 – extracted times of ethanol.

In Fig.4, the surface charts showed that the relation of CAE with the couple of experiment variables, the rest of variable has stably keptin central value level. From the surfaceresponse design (fig.3b), we could determine at the optimal point ofmain factors which made the highest extract yield.

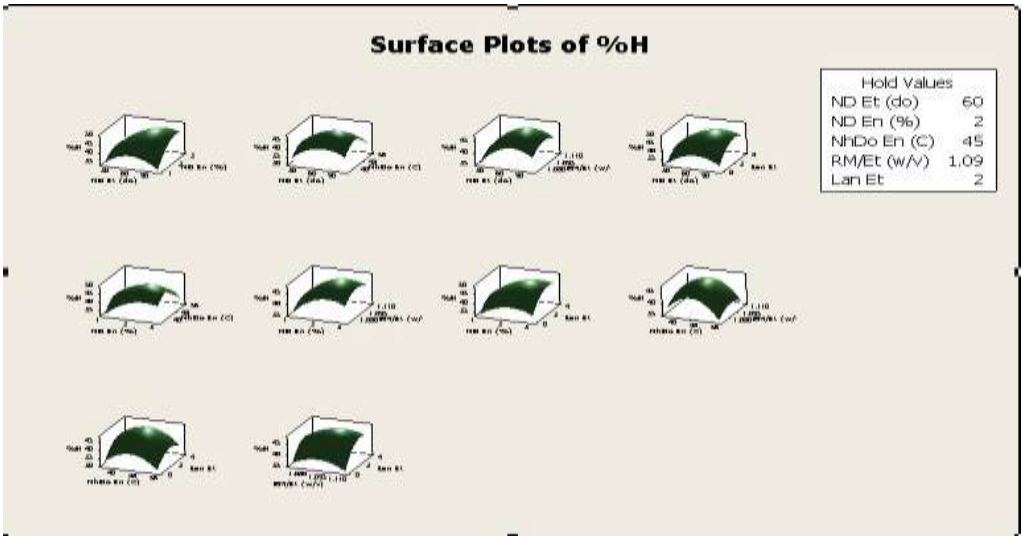

Fig 4. Surface plots

Following the results in fig. 5, The value of variables gave the value of function that the efficiency of collected CA optimized in ethanol 74,9495%, Viscozyme 2,5354%, 45,28280C, centella/ethanol 1,0930 (w/v) and 2,7879 (times of extraction). The CAE yield reached maximum 49,2087%.

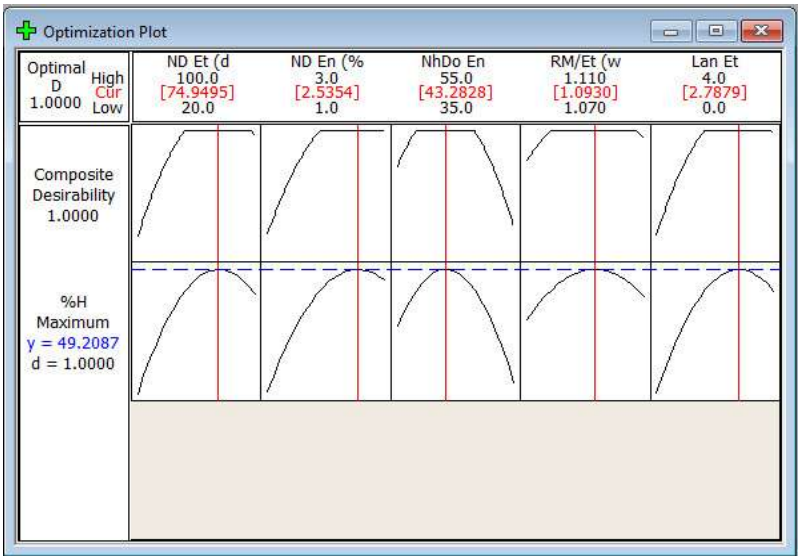

Fig5. Experiment for selecting a D-optimal response surface design

To evaluate the level of the concordance between model and reality, the evaluated experiments was conducted in the found optimal points and compared with the maximum value of targeting function inferred from the model (Y_{max}) with the experimenting value (Y'_{max}) received from the table

Table3. Experimental results in evaluating optimum value

Extracted conditions	**Model**	**Reality**
Ethanol concentration(%)	74,9495	75
Enzyme concentration (%)	2,5354	2,5
Enzyme temperature (^oC)	43,2828	43
Centella/ethanol (w/v)	1,0930	1/9
Times ofethanolextraction (time)	2,7879	3
Y, The efficiency of Centella extract collection (%)	49,2087	48,92±1.25*

*Average value ±Standard deviation
The same charaters in the values are not different (p>0.05)

The equivalent of CAE yield following theory and experiment:

$$\textbf{Equivalent} = \frac{Y'_{max}}{Y_{max}} *100 = \frac{48.92}{49,2087} *100 = 99,41\%$$

These results demonstrated the optimum of the factors that reached the high reliable and meaningful.So, the efficiency of the extracted Centella extract collection following the optimum method reached 48,92%, increasing 4,92% when comparing with the classical extract method (44,20%).Juraiporn Soboonwong *et al.* (2012), asiaticoside is one of three compounds in saponin triterpenoid group that had main effection stimulated biosynthesis collagen through activation of receptors ß-TGF, increasing healing the wound. Nguyen Thi Le Thuy *et al.* (2006) demonstrated that it increased higher when combinating with Melaleuca oil and madecassol reached the highest.

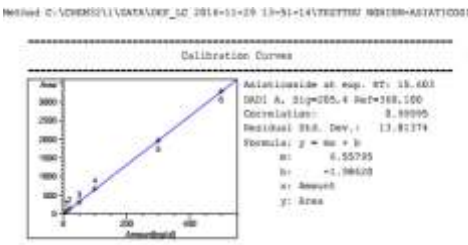

Fig 6. Standard lines of asiaticoside

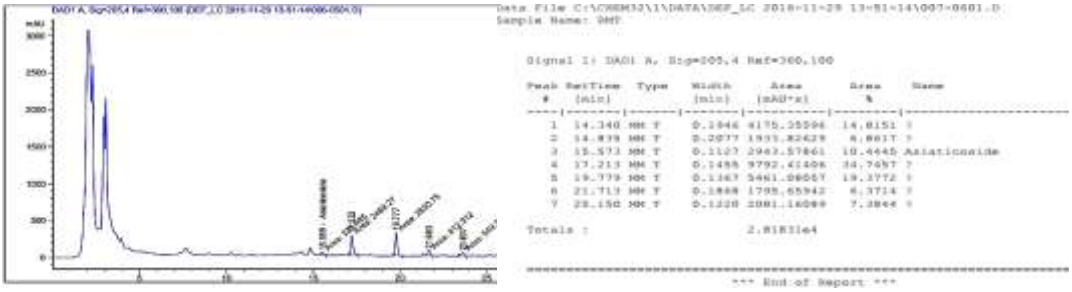

Fig 7. The asiaticoside content of CAE by optimal method

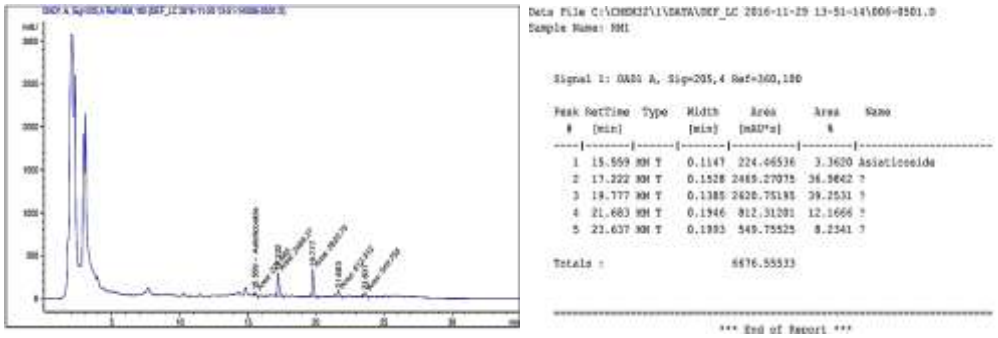

Fig 8. The asiaticoside content of CAE by classical menthod(ethanol 96%)

The content of asiaticoside in CAE has optimized in the optimal process (RMT) reached 37,91mg/mL (fig 7) higher 2,32 times when comparing with the classical method extract (ethanol) (RM1) only reached 16,31mg/mL (fig 8). Besides, when comparing with other studies as: M. H. Rafamantanana *et al.* (2009), by using soxhlet method with methanol solvent 100% in 8 hours, only extracted 1mg/mL asiaticoside, supercritical carbon dioxide extraction method (asiaticoside content around 1,1 - 8,1 mg/g) and the reflux extraction method (16 mg/mL).

IV. Conclusion

The results of the study has determined the optimal conditions for Viscozyme L with increasing the CAE yield with enzyme concentration 2,5 %, enzyme temperature 43^{o}C, ethanol concentration for extracting 75%, Centella/ethanol 1/9 (w/v) and extracting 3 times, the CAE yield higher than 2 times when comparing with the classical extract method (only using ethanol solvent). Besides, the optimal extraction method help to increase the quantity of asiaticoside 2,32 times when comparing with the classical extractmethod.This is the study that can be extended for applying enzyme in the CAEprocessto help cut down the wase of time, temperature and the concentration of solvent extraction.

References

[1] Van Anh Nguyen Thi, Minh Hanh Truong Thi, Nghiêncứucácđiềukiệnchiếttáchasiaticosidetừrau má (*Centella asiatica*). Tạpchí KH và CN - Việnkhoahọcvàcôngnghệ Việt Nam, Vol. 49, No. 6A: 212-219. (2011).

[2] Chew, K. K., ..., & Ho, C.W, Effect of ethanol concentration, extraction time and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of *Centellaasiatica* extracts, International Food Research Journal, 18: 571-578, (2011).

[3] Chippada, S. C., &Vangalapati, M, Antioxidant, an anti-inflammatory and antiarthritic activity of *Centellaasiatica* extracts. J. Chem. Bio. Phy. Sci, Vol.1, No. 2, Sec. B: 260 - 269 (2011).

[4] V. Chi và T. Hợp. CâycỏcóíchViệt Nam, NXB. Giáodục, 509 (1999).

[5] Hamid, A. A., Shah, Z. M., Muse, R., & Mohamed, S, Characterisation of antioxidative activities of various extracts of *Centellaasiatica* (L) Urban, Food chemistry, 77 (4): 465-469, (2002).

[6] Nguyễn ThịHoài, Phânlập acid Asiatic và acid madecasictừraumá (*Centellaasiatica* (L.) Urb. - Apiaceae) thuhái ở ThừaThiênHuế,

TạpchíDượcHọc, Vol. 424, No. 5:22-25 (2011).
[7] H. P. Hoàng, CâycỏViệt Nam, quyển 2, NXB Trẻ, 477 - 479 (2000).
[8] TônNữLiênHương, ...& N. NgọcSương, Thànhphầnhóahọccủacáccâyraumálásen, hydrocotylebonariensisvàhydrocotyle vulgaris, (apiaceae), ở tỉnhtiềngiangvàthànhphốcânthơ, TạpchíKhoahọc, Số 22, Tập B: 190-199 (2012).
[9] Nguyễn ThịHoàng Lan, ...&ĐỗThị Trang, Nghiêncứucôngnghệtríchlytinhdầutừlátíatô, J. Sci. &Devel, Vol. 12, No. 3: 404 - 41 (2014).
[10] Leng, Y. X., ..., and R. J. Yang, Aqueous enzymatic extraction of sunflower seed oil, Food and Fermentation Industries (in Chinese), 32 (10):127-31 (2006).
[11] Đ. T. Lợi, Những cây thuốc và vị thuốc Việt Nam, HàNội, nhàxuấtbản y học, 631-632, (1990).
[12] HuỳnhThịNhã, HuỳnhNgọcOanh, Võ Quang Vinh, Phan PhướcHiền. Nghiêncứứngdụng enzyme Viscozyme L đểcảithiệnchấtlượngtrà Ô long, TạpchíNôngnghiệp&Pháttriểnnôngthôn, số 8: 61-64 (2016).
[13] M.H. Rafamantananaa, E. Rozet, G.E. Raoelisona, K. Cheuk, S.U. Ratsimamanga, Ph. Hubert, J. Quetin-Leclercq, "An improved HPLC-UV method for the simultaneous quantification of triterpenic glycosides and aglycones in leaves of *Centellaasiatica* (L.) Urb (Apiaceae)," Journal of Chromatography B, Vol. 877, No. 23: 2396-2402 (2009).
[14] JuraipornSomboonwong, ...&Mayuree H Tantisira, Wound healing activities of different extracts of *Centellaasiatica* in incision and burn wound models: an experimental animal study, Complementary and Alternative Medicine, 12:103 (2012).
[15] Phùng Thị Sinh, Định lượng các vitamin trongraumábằngphươngphápsắckýlỏngcaoáp, Tạpchí Y Học TP. Ho Chi Minh, Tập 8, Số 1:88-91 (2004).
[16] Nguyễn Thị Linh Tuyền, ĐặngVănGiáp, Nguyễn Minh Đức, Nghiêncứutốiưuhóaquytrìnhchiếtxuấtcaoraumá*Centellaasiatica* (L.) URB. J003 (v), Tạpchí Y Học TP. HồChí Minh, 15 (1): 555-558 (2011).
[17] Nguyễn ThịLệThủy, ...& Phan Kim Ngọc. Thiếtkếmàng gelatin –alginate cócốđịnhthuốcnamứngdụngtrongđiềutrịtổnthươngbỏng, TạpchíPháttriểnKhoahọc&Côngnghệ, Vol. 9, No. 6:37-42 (2006).