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VALIDATION AND DEVELOPMENT OF TLC-DENSITOMETRY METHOD FOR STANDARDIZATION OF SOURSOP LEAF EXTRACT (*ANNONA MURICATA* LINN.) WITH QUERCETIN

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Keywords:

Standardization, Quercetin, Soursop Leaf extract (*Annona muricata,* Linn.), TLC-Densitometry, Validation and development method

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ABSTRACT: Standardization is a process involving various chemical analysis methods based on pharmacological data involving general physical and microbiological analyzes aimed at the safety of a natural extract. To obtain the correct result, the standardization step is preceded by method validation. Quercetin can be obtained from the leaves of soursop (*Annona muricata* Linn.), which use for treatment fever, diarrhea, anti-convulsive, antifungal, antimicrobial, gout, itching, and flu. Determination of quercetin in soursop leaf extract using TLC-Densitometry must be validated by testing selectivity, limit of detection (LOD), limit of quantification (LOQ), linearity, precision, accuracy, and range. Densitometry scanning of the plates silica gel 60 F_{254} directly at 265 nm was used for analysis of quercetin. The result of selectivity test was 1.53 (>1.5) with mobile phase toluene: ethyl acetate: formic acid (4: 3: 0.4; v/v/v), limit of detection (LOD = 0.018 µg), limit of quantification (LOQ = 0.060 µg), linearity y = 9387.3x − 767.39 (r = 0.9996 and Vxo = 1.9%), Coefficient of Variation (C.V.) of precision 1.906525% and the percent recovery of 96.6% ± 8.26%. The validated and development method was applied for standardization of quercetin in soursop leaf extract and resulted in the quercetin assay in soursop leaf extract was 1.288% ± 9.996% (w/w).

INTRODUCTION: Standardization is a process involving various chemical analysis methods based on pharmacological data involving general physical and microbiological analyzes aimed at the safety of a natural extract [1, 2]. To have a good result, there must be a relationship between the quality of the raw materials, in the process materials and the final product; it becomes necessary to develop reliable, specific and sensitive quality control methods using a combination of classical and modern instrumental analytical methods.

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Standardization is an important measure to ensure quality control of herbal medicine[3]. Standardization of herbal medicines is the process of determining the levels compared to the specific standards or characteristics, constant parameters, qualitative and quantitative values that bring true quality assurance, efficacy, safety, and reproducibility. Therefore standardization is a tool in the quality control process [4-7].

Standardization of drugs means the confirmation of identity and the determination of quality and purity [8]. Standardization here emphasizes the determination of the compound content responsible for pharmacological effects, which is a process of ensuring good quality and standardization for medicines and herbal products [1, 9]. To obtain the correct result, the standardization step is preceded by method validation.

Validation of analytical methods is the assessment of an analytical method based on laboratory experiments to demonstrate that the parameters of accuracy, precision, specificity/selectivity, detection limits (LOD), quantification limits (LOQ), linearity, and range meet requirements for users [10]. In the quantitative analysis of components and substances of medicinal materials derived from biological samples such as those in plants must meet the parameters of accuracy, precision, specificity/ selectivity, detection limits (LOD), quantification limits (LOQ), linearity, and range [11]. Quercetin is an active compound that can be obtained on soursop leaves *(Annona muricata,* Linn.) and widely used as an alternative treatment for fever, diarrhea, anti-seizure, anti-fungal, antiparasitic, antimicrobial, lumbago, gout, itching, anticancer, anticonvulsant, antidiabetic, anti-inflammatory, anti-arthritic and flu traditionally [12-20].

The determination of quercetin content can be determined by Spectrophotometric, HPLC, RP-HPLC method [21-25]. The TLC-Densitometry method is the primary choice for the analysis of chemical components derived from plants because it has the flexibility to detect almost all compounds, even some inorganic compounds [26]. Systematic approaches and well-designed methodologies for standardization of raw materials and herbal preparation formulations are now widely developed. Given the growing interest in herbal medicine, standard methods of herbal medicine are developed and used in different formulations [26].

MATERIALS AND METHODS:

Instrument: Analytical Scales (O Haus Pioneer), Rotary Vacuum Evaporator (Heidolph Laborota 4000), 20 × 10 × 5 cm^3 chromatography chamber (CAMAG), Densitometer (Shimadzu Dual Wavelength Chromato Scanner CS 930), UV CAMAG Lamp, Capillary Pipes, and Common Glass Tools used in the chemical laboratory analysis.

Chemicals: The soursop leaves (*Annona muricata,* Linn.) were collected from the Purwodadi Botanical Garden, East Java and were determined by the Indonesian Institute of Sciences UPT of the Purwodadi Botanical Gardens Conservation Center. Quercetin (pharmaceutical grade, Sigma-Aldrich), ethanol pa (Merck), technical acetone, ethyl acetate pa (Merck), toluene pa (Merck), formic acid pa (Merck), methanol pa (Merck), and Silica Gel TLC 60 F254 (Merck).

Procedure:

Making Simplicia: The soursop leaves (*Annona muricata,* Linn.) that have been washed, drained and dried by air-dried in the open air with no direct sunlight, approximately at room temperature of 25-30 °C for 1 week. Samples that have dried in a blender and sieved to form a rather coarse powder.

Making Extract: The 0.65 kg soursop leaf Simplicia was extracted using technical acetone (1:10) in the macerator for 24 h with an occasional shake. Remaceration is at least twice, with the same type and amount of solvent. The extracts were concentrated using a rotary vacuum evaporator at temperatures between 60 - 70 ºC and concentrated extracts.

Determination of Water Content: Weigh carefully 1-2 grams of simplicia and extract in a covered porcelain crust that has been preheated at 105^0 C for an hour and has known the constant weight. The material in the exchange rate is leveled by shaking the exchange rate, dried at 105 ºC for five hours, weighed. The step is repeated until the weight of a constant heating product is obtained (the difference of weighing 0.25% or not exceeding 0.5 mg).

Preparation of Quercetin Standard Solution: Standard solution of parent quercetin was prepared in ethanol p.a 1000 µg/ml. A standard solution of quercetin (60 - 600 µg/ml) was prepared in an ethanol solvent p.a.

Determination of Selected Wavelength: In the TLC plate, bottles of soursop leaf extract (*Annona muricata* Linn.), quercetin solution, soursop leaf extract (*Annona muricata* Linn.) were added to quercetin solution. Next observed the spectra in the wavelength region of ultraviolet.

Validation Method:

Selectivity: Bottled two µl quilled standard quercetin solution quercetin 300 µg/ml, a solution of 300 mg soursop leaf extract (*Annona muricata,* Linn.) in 10.0 ml ethanol p.a, 1.0 ml solution of sample solution which has been additions with 1.0

ml of standard quercetin 300 µg/ml on 60 silica gel TLC plate F254. Eluate of TLC plate with mobile phase toluene: ethyl acetate: methanol (4:0.5:0.5), toluene: ethyl acetate: formic acid (7:3:1), toluene: ethyl acetate: formic acid (4:3:0,4) then observed its chromatogram with Densitometer and calculated its degree of resolution (R_s).

Limit of Detection (LOD) and Limit of Quantitation (LOQ): Bottled two µl quercetin solution of 60 - 500 µg/ml and bottle some ethanol p.a. on the silica gel TLC plate 60 F_{254}. Elution the TLC plate with mobile phase toluene: ethyl acetate: formic acid (4: 3: 0.4) and then observed the area by Densitometer at selected wavelengths. Calculate the Standard Deviation (SD) area of the spot ethanol p.a., and slope regression equation of quercetin standard solution of 60 - 500 µg/ml. Calculation of detection limits (LOD) and quantity limits (LOQ) with the following formula [10]:

$$Q = k \times S_b / Sl$$

Q = LOD or LOQ
k = constant value, (LOD = 3, LOQ = 10)
Sb = SD blank
Sl = slope of regression equation quercetin

Linearity: Bottled two µl standard solution of quercetin 90-500 µg/ml on plate 60 silica gel TLC 60 F_{254}. Elution of the TLC plate with mobile phase toluene: ethyl acetate: formic acid (4:3:0.4) and then observed the area at a selected wavelength with Densitometer.

Precision: Bottled two µl standard solution of 200 µg/ml quercetin some of the bottling points on the 60 F_{254} silica gel TLC plate. Elution of the TLC plate with mobile phase toluene: ethyl acetate: formic acid (4: 3: 0.4). Observed area of the selected wavelength using Densitometer then calculated the mean area, Standard Deviation (SD), and Coefficient of Variation (KV).

Accuracy: One series of quercetin with a weight increase of 80-120% (2.4 mg, 3.0 mg and 3.6 mg), added 0.5 ml, 1.0 ml, 2.0 ml, 3.0 ml, 4, 0 ml of 1.5 grams of soursop leaf extract (*Annona muricata* Linn.) in 50.0 ml ethanol p.a. It is then bottled on the silica gel 60 F_{254} TLC plate of two µl. Elution of the TLC plate with mobile phase toluene: ethyl acetate: formic acid (4: 3: 0.4). The measured area at a selected wavelength and determined (%) Recovery.

Determination of Quercetin Levels in Extracts: From one ml of 300 mg soursop leaf extract (*Annona muricata* Linn.) in 10.0 ml ethanol p.a. Then it was added that 1.0 ml of quercetin standard solution with increased concentration (90-500 µg/ml), was bottled on the silica gel 60 F_{254} TLC plate of two µl. Elution on the TLC plate with mobile phase toluene: ethyl acetate: formic acid (4: 3: 0.4). Measured area and determined based on the calibration curve of the quercetin standard solution.

RESULT:

Extract Soursop Leaf (*Annona muricata* Linn.): From 2.I kg (wet weight) soursop leaves (*Annona muricata* Linn.) obtained 650 g of dried leaves (31.0%). From 650 g of dried leaves macerated with 12 liters of acetone and after concentration with rotary vacuum evaporator obtained 53.7189 g of soursop leaf extract (*Annona muricata* Linn.) (8.26%).

Determination of Water Content: The water content in soursop leaf (*Annona muricata* Linn.) is 13.78 ± 0.2060% (w/w). While the moisture content of soursop leaf extract (*Annona muricata* Linn.) is 8.38 ± 0.0907% (w/w).

Determination of Selected Wavelength: From **Fig. 1**, the selected wavelength on the determination of quercetin levels is 265 nm.

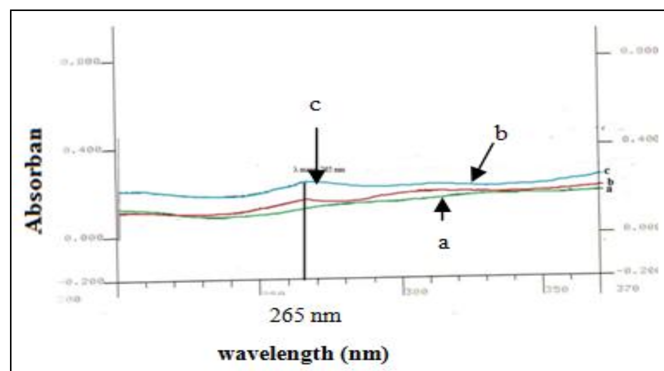

FIG. 1: SPECTRA (A) SOURSOP LEAF EXTRACT (B) QUERCETIN SOLUTION (C) SOURSOP LEAF EXTRACT ADDED QUERCETIN

Validasi Metode:

Selectivity: Selectivity test results determined from the value of the degree of resolution can be seen in **Table 1**.

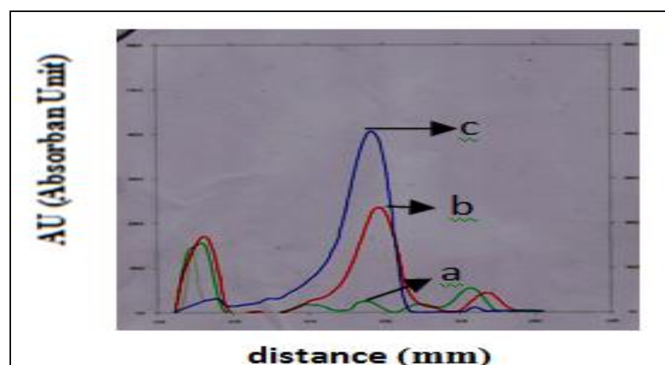

FIG. 2: CHROMATOGRAM (A) LEAF EXTRACT OF SOURSOP (B) QUERCETIN SOLUTION (C) SOURSOP LEAF EXTRACT OF ADDED QUERCETIN IN MOBILE PHASE TOLUENE: ETHYL ACETATE: METHANOL (4:3:0.4)

TABLE 1: DEGREE OF RESOLUTION (Rs)

Mobile phase	R_s	
	R_{s1}	R_{s2}
toluene: ethyl acetate: methanol (4: 0.5: 0.5)	0,57	1.57
toluene: ethyl acetate: formic acid (7:3:1)		
toluene: ethyl acetate: formic acid (4:3:0.4)	1,52	1,53

By looking at the chromatogram **Fig. 2** and the degree of resolution **Table 1**, a toxic toluene phase is chosen: ethyl acetate: formic acid (4: 3: 0.4) because of its resolution degree ≥ 1.5 [27, 28].

LOD and LOQ: The regression equation of quercetin solution is 60 - 500 µg/ml, y = 10492x - 152.99 y and S.D of ethanol p.a p.a is 63.4825.

LOD = 3 × 63.4825/10492 = 0.018 µg

LOQ = 10 × 63.4825/10492 = 0.060 µg

Linearity: Linearity test results obtained y = 9387.3 x-767.39 (r = 0.9996), which can be explained there is a linear relationship between quantity quercetin with the area.

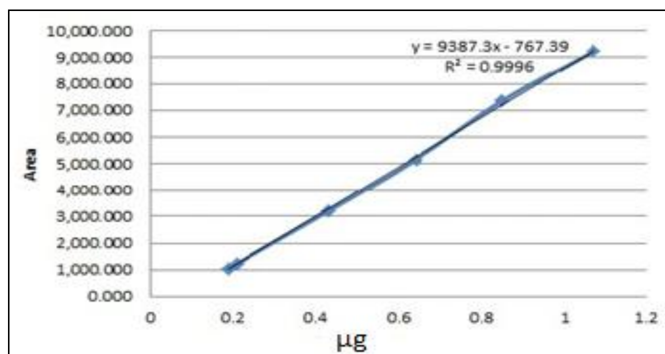

FIG. 3: LINEARITY OF QUERCETIN SOLUTION

Precision: The quercetin solution of 213.6 µg/ml was bottled eight times with a two-µl bottling volume with a spacing of 1 cm each on the silica gel 60 F_{254} TLC plate. Precision test results obtained the average area of quercetin is 3129.575 with Coefficient of Variation (C.V) = 1.906525% (C.V. requirement <2%) [27-28].

Accuracy: The accuracy of the yield (%) recovery of quercetin by adduct can be seen in **Table 2**. Retrieved (%; w/w) recovery 96.6 ± 7.99 (required for biological samples 80-120%) [27-28].

TABLE 2: ACCURACY

Known quercetin (mg)	Replication	Quercetin obtained (mg)	(%, w/w) Recovery
2.4 (80%)	1	2.5839	107
	2	2.2745	94.8
	3	2.1754	90.6
3.0 (100%)	1	2.4543	81.8
	2	2.7730	92.4
	3	2.8708	95.7
3.6 (120%)	1	3.8056	106
	2	3.6558	102
	3	3.5781	99.4
average			96.6
Standard Deviasi (S.D)			7.99
The coefficient of Variation (C.V.)			8.26

Determination of Quercetin Levels in Extracts: After obtaining optimal validation conditions, it was applied to standardize the determination of quercetin content in soursop leaf extract **Table 3**.

TABLE 3: DETERMINATION OF QUERCETIN LEVELS IN SOURSOP LEAF EXTRACTS (*ANNONA MURICATA* LINN.)

Repli-cation	Water content (%, w/w)	Weight weighed (mg)	Weight gain (mg)	quercetin levels % (w/w)
1	8.38	300.1	3.486	1.162
2	8.38	300.1	3.848	1.282
3	8.38	300.1	4.258	1.419
average				1.288
Standard Deviation (S.D)				0.1287
The coefficient of Variation (C.V.)				9.996

Standardization on the determination of quercetin content in soursop leaf extract obtained 1.288 ± 0.1287% (w/w).

DISCUSSION: The choice of quercetin wave-length was obtained at 265 nm, another researcher reported 266 nm [21]. This wavelength difference of one nm can be considered the same. Selectivity test aims to determine the phase of motion selected with the parameter of the degree of resolution.

In this study selected phase of motion was toluene: ethyl acetate: formic acid (4: 3: 0.5), because the degree of resolutions was > 1.5 [27-28]. Other researchers used the mobile phase toluene: ethyl acetate: methanol (4: 0.5: 0.5) obtained a resolution value of 0.57 and 1.57 [29]. This use of mobile phase of toluene: ethyl acetate: formic acid is similar [30] because the ratio is less precise, it is developed by changing the ratio of the mobile phase system to toluene: ethyl acetate: formic acid (4: 3: 0.5). Different mobile phase systems will also differ the polarity, where the separation process depends on the polarity of the mobile phase system that affects the value of the degree of resolution.

The linearity test was performed by bottling the standard quercetin solution of 90 - 500 µg/ml by two µl (0.12-1.00 µg) on the silica gel 60 F_{254} TLC plate, eluted and then measured the area. From the calculation results obtained regression equation y = 9387.3x - 767.39 (r = 0.9996). It can be explained that there is a linear relationship between quercetin with bottle and area. The linearity of other researchers in the range of weight of quercetin 0.1-1.00 µg and generated regression equation y = 2.8437x + 202.43 (r = 0.9881) [31].

From the results of calculations in this study, obtained limit of detection (LOD) and limit of quantities (LOQ) that is 0,018 µg and 0,060 µg. In research [31], the limit of detection (LOD) and limit of quantification (LOQ) for quercetin compounds were 0.05429 µg and 0.16451 µg. The difference of LOD/LOQ depends on the researcher's thoroughness so that if obtained by LOD/LOQ is small it will be obtained more sensitive observation. Precision in this study obtained Coefficient of Variation (C.V.) of 1.91% (C.V. Requirements <2%). Other research [31], obtained the C.V. of 6.53%. This difference occurs because of differences in the expertise of researchers, resulting in different precision [10].

Accuracy is done by addition method because the matrix of artificial nature sample cannot be made. The accuracy test is used to show the percentage of the proximity of the analysis results with the actual quercetin content [10]. The accuracy of quercetin in this study obtained the result of 96.6 ± 8.26% (w/w), this result has met the requirements of the accuracy of bioanalysis 80-120% and precision (C.V.) <10% [32]. The result of accuracy is not much different as reported [19], obtained an average percentage recovery of quercetin of 98.6%.

Application of TLC-Densitometry method for determination of quercetin content in soursop leaf extract *(Annona muricata* Linn.) was performed after the optimal optimum condition was obtained. From the result of the determination of quercetin content in soursop leaf extract 1.288 ± 9.996% (w/w). Reported [33], the content of the selected flavonoid as a quercetin compound in soursop leaf extract is (9.96 ± 1.53) mg/g. Differences in quercetin levels are caused by differences in sampling sites, genetic factors, growing environment, treatment during growth, and different harvest time [34-35].

CONCLUSION: Standardization of soursop leaf extract (*Annona muricata* Linn.) contains quercetin 1.288 ± 0.1287% (w/w).

RECOMMENDATION: The TLC-Densitometric method can be used to standardize soursop leaf extracts containing quercetin.

CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

REFERENCES:

1. Kunle OF, Egharevba HO and Ochogu P: Standardization of herbal medicines- A review; International Journal of Biodiversity and Conservation 2012; 4(3): 101-12.
2. Ahmed S and Hasan MM: Standardization of crude drugs: a precise review. World Journal of Pharmaceutical Research 2015; 4(10): 155-174.
3. Kadian N, Raju KSR, Rashid M, Malik MY, Taneja I and Wahajuddin M: Comparative assessment of bioanalytical method validation guidelines for the pharmaceutical industry. Journal of Pharmaceutical and Biomedical Analysis 2016; 126: 83-97. Doi.org: 10.1016/j.jpba.2016.03.052
4. Kumari R: A Review on the Standardization of herbal medicines. International Journal of Pharma Sciences and Research 2016; 7(2): 97-106.
5. Butt J, Ishtiaq S, Ijaz B, Mir AA, Arshad S and Awais S: Standardization on herbal formulations at molecular level. Journal of Pharmacognosy and Natural Products 2016; 4(1): 1-8. Doi: 10.4172/2472-0992.1000150
6. Shulammithi R, Sharanya M, Tejaswini R and Kiranmai M: Standardization and quality evaluation of herbal drugs. IOSR Journal of Pharmacy and Biological Sciences 2016; 11(5): 89-100. Doi: 10.9790/3008-11050189100
7. Patwekar SL, Arvind BS, Manoj SG, Snehal RP and Ashwini PP: Standardization of herbal drugs: An overview. The Pharma Innovation 2015; 4(9): 100-4.
8. Pandey A and Tripathi: Concept of standardization, extraction and pre phytochemical screening strategies for the herbal drug; Journal of Pharmacognosy and Phytochemistry 2014; 2(5): 115-9.

9. Sachan AK, Vishnoi G and Kumar R: Need of standardization of herbal medicines in the modern era. International Journal of Phytomedicine 2016; 8: 300-7. Doi: 10.5138/09750185.1847
10. Harmita and Perhitungannya PPVMDC: Majalah Ilmu Kefarmasian 2004; I(3): 117-35.
11. United States Pharmacopeial Convention. The United States Pharmacopeia 36-National Formulary 31 (USP36-NF31). 34st Edition. Rockville USA: United States Pharmacopeial Convention Inc. 2013.
12. Moghadamtousi SZ, Fadaeinasab M, Nikzad S, Mohan G, Ali HM and Kadir HA: Review: *Annona muricata* (Annonaceae): A review of its traditional uses, isolated acetogenins and biological activities. International Journal of Molecular Sciences 2015; 16: 15625-58.
13. Patel S and Patel JK: A review on miracle fruit of *Annona muricata*. Journal of Pharmacognosy and Phytochemistry 2016; 5(11): 137-48.
14. Abbas SI, Mohammed MT and Al-Mahdi RA: Identification of some *Annona muricata* L. (Soursop) components and their antioxidant effect in rats. The Iraqi Postgraduate Medical Journal 2015; 14(4): 576-80.
15. Agu KC, Okolie NP, Eze I, Anionye JC and Falodun A: Phytochemical analysis, toxicity profile, and hemomodulatory properties of *Annona muricata* (Soursop). Egyptian Journal of Haematology 2017; 42(1): 36-44. Doi: 10.4103/1110-1067.206431
16. Nawwar M, Ayoub N, Hussein S, Hashim A, El-Sharawy R, Wende K, Harms M and Lindequist U: A Flavonol triglycoside and investigation of the antioxidant and cell stimulating activities of *Annona muricata* Linn. Archives of Pharmacal Research 2012; 35(5): 761-7. Doi: 10.1007/s 12272-012-0501-4
17. Najmuddin SSUF, Alitheen NB, Hamid M and Rahman NA: Comparative study of antioxidant level and activity from leaf extracts of *Annona muricata* Linn. obtained from different locations. Tropical Agricultural Science 2017; 40(1): 119-30.
18. Akomolafe SF and Ajayi OB: A Comparative study on antioxidant properties, proximate and mineral compositions of the peel and pulp of ripe *Annona muricata* (L.) fruit. International Food Research Journal 2015; 22(6): 2381-8.
19. Vaghela BD, Bhavsar D and Jain NK: Effect of aqueous extract of *Annona muricata* (Graviola tea) on the oxidative stress produced by the artificial UV source *in-vivo* and *in-vitro*. International Journal of Current Microbiology and Applied Sciences 2016; 5(10): 648-55. Doi.org:10.20546/ ijcmas.2016.510.072
20. Batubara I, Achmadi SS and Nurwendari W: Extraction technique to separate kaempferol from Soursop (*Annona muricata*) leaves. Journal of Chemical and Pharmaceutical Research 2015; 7(9S): 106-10.
21. Tokusoglu O, Unal MK and Yildirun Z: HPLC-UV and GC-MS Characterization of the flavonol aglycons quercetin, kaempperol and myricetin in tomato pastes and other tomato-based products, Acta Chormatographica 2003; 13: 196-207.
22. Saddiqe Z, Naeem I, Maimoona A, Patel AV and Hellio C: Assay of flavonoid aglycones with HPLC in four species of genus Hypericum. Journal of Medicinal Plants Research 2011; 5(9): 1526-30.
23. Mir SA, Ahangar AA and Bhat AS: Spectrophotometric assays for flavonoids diosmin, quercetin, rutin and morin with Copper, Molybdenum, Lead and Tungsten. International Journal of Pharm Tech Research 2013; 5(2): 383-90.
24. Sanghavi N, Bhasale SD and Malode Y: RP-HPLC method development and validation of quercetin isolated from the plant *Tridax procumbens* L. Journal of Scientific and Innovative Research 2014; 3(6): 594-7.
25. Tuszynska M: Validation of the analytical method for the determination of flavonoids in broccoli; Journal of Horticultural Research 2014; 22(1): 131-40.
26. Bele AA and Khale A: Standardization of herbal drugs: an Overview; International Research Journal of Pharmacy 2011; 2(12): 56-60.
27. Pyka A, Buddzisz M and Dolowy M: Validation Thin Layer Chromatography for the determination of acetaminophen in tablets and comparison with a Pharmacopeial method. BioMed Research International 2013; 1-10. DOI.org:10. 1155/2013/545703.
28. Dolowy M, Kulpinska-Kucia K and Pyka A: Validation of a Thin Layer Chromatography for the determination of hydrocortisone acetate and lidocaine in a pharmaceutical preparation. The Scientific World Journal 2014; 1-10. DOI.org: 10.1155/2014/107879.
29. Chandrappa CP, Govindappa M, Kumar ANV, Channabasava R, Chandrasekar N, Umashankar T and Mahabaleshwara K: Identification and separation of quercetin from ethanol extract of *Carmona retusa* By Thin Layer Chromatography and High-Performance Liquid Chromatography with diode array detection. World Journal of Pharmacy and Pharmaceutical Sciences 2014; 3(6): 2020-9.
30. Rohini RM and Das AK: Determination of lupeol, β-sitosterol and quercetin from ethyl acetate extract of Rhizophora mucronata bark by HPTLC technique. Asian Journal of Pharmaceutical and Clinical Research 2011; 4(1): 103-5.
31. Joshi C, Savai J, Varghese A and Pandita N: Validated HPTLC fingerprint analysis for simultaneous estimation of quercetin, kaempferol and asatic acid in leaves of two different chemotypes of *C. asiatic*. International Journal of Pharmaceutical and Biological Research 2012; 3(5): 202-12.
32. Wang Y, Cao J, Jian-Huang W, and Zeng S: Simultaneous determination of quercetin, kaempferol and isorhamnetin accumulated human beast cancer cells, by High-Performance Liquid Chromatography. Journal of Pharmaceutical and Biomedical Analysis 2005; 39: 328-33.
33. Pieme CA, Kumar SG, Dongmo MS, Moukette BM, Boyoum FF, Ngogang JY and Saxena AK: Antiproliferative activity and induction of apoptosis by *Annona muricata* Linn. (Annonaceae) Extract on human cancer cells. BMC Complementary and Alternative Medicine 2014; 1-10.
34. Haghighi NZ and Salehi H: Comparison between the growth and development of some cultured plants among the different region of Kish Island. Journal of Central European Agriculture 2013; 14(2): 564-76. Doi: 10.5513/JCEA01/ 14.2.1238.
35. Mustafa G, Arif R, Atta A, Sharif S and Jamil A: Bioactive compounds from medicinal plants and their importance in drugs discovery. Matrix Science Pharma 2017; 1(1): 17-26.

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