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EFFECT OF SAMPLE PREPARATION AND TLC METHODS ON THE QUANTITATION OF QUERCETIN CONTENT IN ASTHMA WEED

Rajalakshmi Padma Vairavasundaram[1]*., Kalaiselvi Senthil[2]

[1]Lecturer, Vivekanandha college of Arts Sciences For women, Tiruchengode, Tamilnadu, India.

[2]Lecturer, Department of Biochemistry and Biotechnology, Avinashilingam University for Women, Coimbatore- 641043.

E mail address: pvrajivicas@yahoo.co.in

ABSTRACT

Flavonoids compounds have anti-oxidation properties in that they remove free radicals, reduce blood pressure and reduce vessel thrombosis. So many extraction methods are available, but the selection of specific solvent system for the separation and quantification of quercetin is important. It can be easily analyzed by chromatographic methods, but before testing a new type of plant material it is opportune to optimize chromatographic conditions. The aim of this study is to optimize the chromatographic conditions in TLC of flavonoids, as standard compounds that may be present in Euphorbia hirta. We compared 10 different mobile phases, to find the most appropriate mobile phase. The results showed that the optimal extraction parameters were as followings: concentration of methanol solution was 60%, with ethylacetate (40%).

Introduction

Natural products provide a rich source of bioactive molecules used for treating a wide range of different human diseases [1]. Even in light of new methodologies for screening large, diversity oriented small molecule libraries, natural products still provide a large number of lead compounds used for developing new drugs [2]. Natural product drugs include aromatic polyketides, polyethers, coumarins, flavonoids, terpenoids, alkaloids, and aminoglycosides. [3]

In a single species, dozens of different flavonoids may be present. Of the several hundred aglycones isolated from plants, only eight are distributed widely [4]. The eight most common flavonoid nuclei are the flavonols kaempferol, quercetin and myricetin, the anthocyanidins pelargonidin, cyanidin and delphinidin, and the flavones apigenin and luteolin.

Euphorbia hirta, (family-Euphorbiaceae) is an herb called as Asthma weed found in many parts of the world. In Sanskrit it means "Dugadhika" According to the Doctrine of Signatures, the plant has a reputation for increasing milk flow in women, because of its milky latex, and is used for other female complaints as well as diseases of the respiratory tract. The plant has been reported as increase in urine output, antidiarrheal, antispasmodic, anti-inflammatory etc. [5] Its still increasing use led to the need for standardization and analysis of this natural product. The analysis is easily performed by chromatographic methods – thin layer chromatography, gas chromatography and

** Corresponding Author: Ms.. P. V. Rajalakshmi, Lecturer, Department of Biochemistry, Vivekanandha College of Arts & Sciences for Women, Elayampalayam, Tiruchengode. Tamilnadu, India. Phone: +919443420376.*

high-performance liquid chromatography. To identify a group of compounds using these methods, it is necessary to have a set of standard substances for comparison of the analysis results of the unknown compound. The mobile phase for thin layer chromatography optimization process proved to be able to provide accurate, precise and reproducible method of characterization and analysis of chromatographic parameters.

In our work we tested the efficacy of seven solvents appropriate for extraction of flavonoids especially quercetin. At present there are no scientific reports on the extraction of quercetin from the aerial parts of Euphorbia hirta. In this study, the optimal solvent systems for the separation of quercetin from euphorbia hirta were investigated systematically in order to explore a proper process to utilize the E.hirta plant in the area of drug development.

Materials and Methods

Plant material

The plant was collected from the garden of Vivekanandha college of Arts & Sciences for women, tiruchengode, Tamilnadu, India and the plant was identified, confirmed by Botanical Survey of India (BSI), Southern Circle, Coimbatore, India and a voucher specimen (No. DBC 001) was deposited at Department of Biochemistry, Vivekanandha college of Arts & Sciences for women, tiruchengode, Tamilnadu, India

Extraction process

The main factor that affects the extraction process of flavonoids is solvent and their ratios were studied individually. Extraction of flavonoids from E.hirta leaves, stem, roots and whole plant using Petroleum ether, Benzene, Acetone, Methanol, Ethanol, Methanol: Ethylacetate (80:20), and Methanol: Ethylacetate (60:40) was carried out.

Identification of flavonoids by thin layer chromatography (TLC)

The analysis was performed on precoated 20 _ 20 cm (0.25 mm thick) TLC plates K6F Silica gel 60 A purchased from Whatman, USA. 10 microlitre of standard quercetin solution (concentration 0.1 mg ml–1), was applied as spots onto TLC sheets. Ten different mobile phases (Table I) were selected (according to their polarity) to establish the *R*F value for every standard (all solvents were of analytical grade). The plates were developed at room temperature in a vertical separating chamber to the height of approximately 16 cm from the start. The chamber was previously saturated with the appropriate mobile phase (saturation time was 1 hour). After drying, visualization was performed in two ways:

i) in short UV light (254 nm)
ii) spraying with 1% ferric chloride - 1% potassium Ferricyanide mixture (1:1)

Table: 1 Thin layer chromatographic solvent sytems

S. No	Solvent System	Ratio
1	Toluene: ethylacetate: acetic acid	30:40:5
2	Petroleum ether: ethyl acetate: Formic acid	30:10:10
3	Toluene: ethylacetate: formic acid	35:10:10
4	Toluene : acetic acid	40:20
5	Cyclohexane:ethylacetate:formicacid	30:12:18
6	Cyclohexane:ethylacetate:aceticacid	31:14:5
7	Tolene:acetone:Formicacid	30:10:10
8	n-Hexane:ethylacetate:aceticacid	31:14:5
9	n-Hexane:ethylacetate:formicacid	31:14:5
10	Carbontetrachloride:acetone:formicacid	30:10:10

Estimation of Quercetin

The aluminum chloride colorimetric method was modified from the procedure reported by [6]. Quercetin was used to make the calibration curve. Ten milligrams of quercetin was dissolved in 80% ethanol and then diluted to 25, 50 and 100 µg/mL. The diluted standard solutions (0.5 mL)

were separately mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank. Similarly, extracts of plant parts were reacted with aluminum chloride for determination of flavonoid content as described above. Extracts were dissolved in 100 ml ethanol and from that 1 ml is taken for the study and represented as microgram of quercetin per gram of the extract.

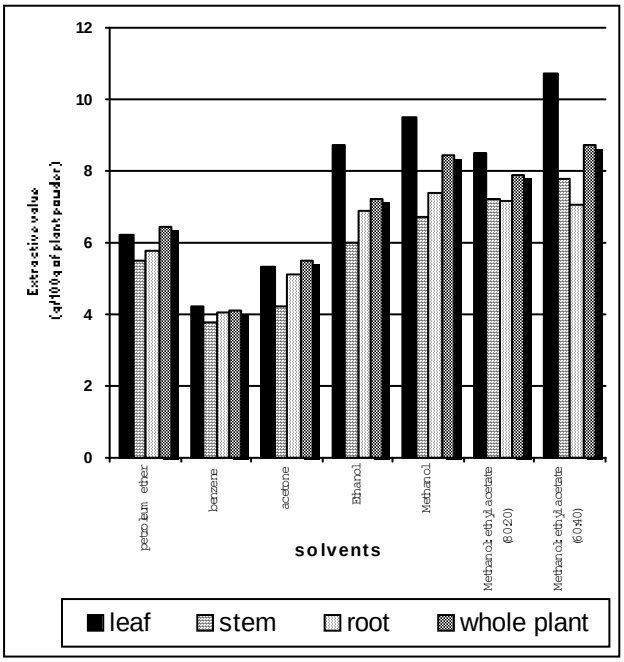
Results and Discussion

To date, over 6000 naturally occurring flavonoids have been isolated and identified; many are common in higher plants[7]. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc. *i.e*. any part of the plant may contain active components.

Effect of solvents on the extractive value

The result of Figure 1.showed the extractive value of each solvent tended to increase gradually with a rise in nonpolar to polar solvent range. A decrease in extraction value of solvents was noticed further more, that is in beyond polar solvent. The goal of every extraction process is rapid and effective isolation of compounds from a matrix by use of a minimum amount of solvent. Traditional methods, for example percolation, exhaustive Soxhlet extraction, or direct extraction with boiling solvent under reflux are most often used [8]. Considering this, among various solvents, Methanol ethlacetate fraction was selected as a best choice for the extraction. Various solvent system used exhibited different effect in changing the fluid polarity and thus had diverse effect on the solubility enhancement of the flavonoids [9]. The optimal extraction valve might be fulfilled when the polarity of the fluid and its quercetin content were coincident. In this study, the results indicated that the optimal solvent system for the extraction of flavonoids was found to be Methanol:ethylacetate (60:40).

Figure.1. Effect of solvents on extractive value of aerial parts of Euphorbia hirta



Thinlayer chromatographic analysis

A data set of *R*F values for the separation of quercetin that may be present in Euphorbia hirta was analyzed by the use of nine different mobile phases. Table II represents the Rf value for the investigational compound quercetin and plant. After determination and comparison of Rf values for quercetin in all the TLC solvent systems, the one with near by Rf value is considered to be the best. Among these 10 solvent systems with Rf value difference less than 0.03, the most suitable TLC system for separating the quercetin was system number 4 – Toluene : Aceticacid, and 7- Tolene : acetone: formic acid.

Table II: RF values of standard Quercetin and aerial parts of *E.hirta*

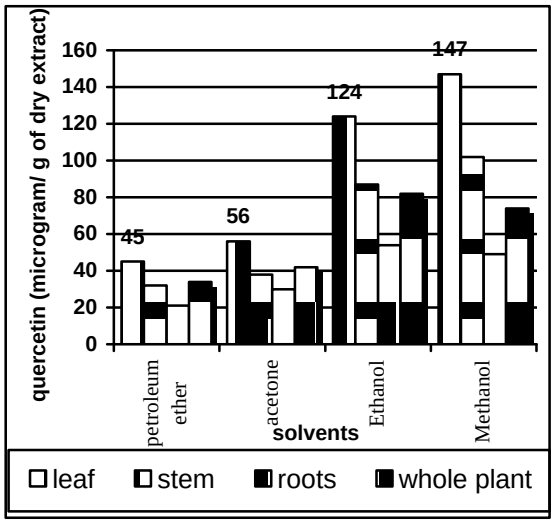
Solvent system	Solvent system	Standard Quercetin	Leaf	Stem	Root	Whole plant
1	Toluene: ethylacetate: acetic acid	0.62	0.60	0.58	0.54	0.58
2	Petroleum ether: ethyl acetate: Formic acid	0.60	0.630	0.65	0.56	0.56
3	Toluene: ethylacetate: formic acid	0.60	0.701	0.72	0.74	0.68
4	Toluene : acetic acid	0.86	0.85	0.84	0.82	0.83
5	Cyclohexane:ethylacetate:formicacid	0.36	0.34	0.30	0.30	0.32
6	Cyclohexane:ethylacetate:aceticacid	0.56	0.6	0.62	0.59	0.58
7	Tolene:acetone:Formicacid	0.68	0.66	0.63	0.60	0.64
8	n-Hexane:ethylacetate:aceticacid	0.74	0.70	0.64	0.69	0.61
9	n-Hexane:ethylacetate:formicacid	0.51	0.48	0.46	0.44	0.49
10	Carbontetrachloride:acetone:formicacid	0.56	0.52	0.54	0.50	0.53

The principal task of chromatography is the separation of mixtures of substances. By "optimization" of the chromatographic process, we mean enhancement of the quality of the separation by changing one or more parameters of the chromatographic system. An ability to foresee, correctly, the direction and scope of these changes is the most important goal of each optimization procedure. [10]

Quercetin content

The quercetin profile in different plant parts, like leaves, stem, roots etc were analyzed (table). The methanolic ethyl acetate fraction of leaves had highest quercetin glycoside content (184microgram/g of dry extract) compared to stem (128microgram/g of dry extract) and roots (68 microgram/g of dry extract). The qualitative and quantitative composition of extracts obtained by a variety of solvents was different, however. Petroleum ether fraction was showed the lowest level of extraction of quercetin than other solvents. Previous reports for extraction of quercetin, a flavonol glycoside from the plant of ***Coriundrum sativum*** [11] revealed that optimum conditions were 30 mL of 45% aqueous methanol, containing 1.85 M HCl, refluxed for 2 hours at 84°C. Quercetin extraction from root and whole plant reported in this study also ethanol was the higher extractive solvent. For quercetin content and extractive value the highest yields were obtained by methanol extraction. For the other solvents the extraction yields for quercetin were smaller. Methanol: ethylacetate (60:40) solvent given the highest extraction yields.

Figure.2. Effect of plant (*E.hirta*) organ on the content of Quercetin



Conclusion

Flavonoids (rutin, quercetin and some unidetified flavonoid-glycosides) are deteminated in the investigated extracts by TLC analysis; Quercetin is present in methanol extract and Methanol:ethylacetate (60:40). A rapid, simple, accurate and specific TLC method for quantitative estimation of quercetin present in the dried plant organs of Euphorbia hirta has been developed.

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Author Information: Dr. **Rajalakshmi Padma Vairavasundaram is working as [1]Lecturer, Vivekanandha college of Arts Sciences For women, Tiruchengode, Tamilnadu, India.**

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