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SPECTROPHOTOMETRIC QUANTIFICATION OF FLAVONOID CONTENT IN HERBAL DRUGS EXTRACTS AND OPTIMIZATION OF MICROWAVE ASSISTED EXTRACTION TECHNIQUE BY USING DIFFERENT SOLVENTS.

Dr. Mohini Kuchekar*[1], Mohini Upadhye[2] and Swati Chawan[3]

[1]Department of Pharmacognosy, P. E. S's Modern College of Pharmacy, Savitribai Phule Pune University, Nigdi, Pune, Maharashtra, India.

[2,3]Department of Pharmacognosy, P. E. S's Modern College of Pharmacy (For Ladies), Savitribai Phule Pune University, Moshi, Pune, Maharashtra, India.

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***Corresponding Author**
Dr. Mohini Kuchekar
Department of Pharmacognosy, P. E. S's Modern College of Pharmacy, Savitribai Phule Pune University, Nigdi, Pune, Maharashtra, India.

ABSTRACT

The quantitative determination of flavonoids in beans, leaves, roots, peels of *Ficus glomerata*, *Citrus aurantium*, *Spinacea oleracea*, *Camellia sinensis* and *Phaseolus vulgaris* respectively was carried out using spectrophotometric methods. Quercetin reagent was used as the standards for calibration of the flavonoids. The plant material was collected from local Pune, Maharashtra, India. In the present study the flavonoid content of ethanolic and aqueous roots, peels, leaves, beans extracts was evaluated by Aluminium chloride colorimetric method. Total flavonoid content in aqueous and ethanolic extract of *Ficus glomerata* (roots extract), *Citrus aurantium* (peels extract), *Camellia sinensis* (leaf extract), *Spinacea oleracea* (leaf extract) and *Phaseolus vulgaris* (seed extract) were found to be (169mg/gm, 623mg/gm), (52mg/gm, 102mg/gm), (126mg/gm, 208mg/gm), (520mg/gm, 458mg/gm) and (262mg/gm, 456mg/gm) respectively. The optimization of extraction conditions were performed using different solvents. It was concluded that ethanolic extract by microwave synthesis gives maximum amount of flavonoid content.

KEYWORDS: Flavonoid content, *Ficus glomerata*, *Citrus aurantium*, *Camellia sinensis*, *Spinacea oleracea*, *Phaseolus vulgaris*.

INTRODUCTION

The extraction and characterization of several active phyto-compounds factories have given birth to some high activity profile drugs [Mandal et al., 2007]. Now days there is raising demand for novel extraction techniques to get maximum quantity of extract with good quality. The reduction of extraction time when powder sample expose to microwaves is an advantage of use of microwave assisted extraction techniques. In this method temperature gradient is kept minimum and it accelerates the heating speed. Additionally microwave assisted extraction allows significant reduction in organic solvent consumption during extraction process [Eskilsson et al., 2000].

The microwave assisted extraction technique was developed and optimized to extract withanolides from *Withania somnifera*, *Cryptotaenia japonica Hass* and *Zingiber officinale* within short period [Jyothi et al., 2010; Kaufmann et al., 2001; Lu et al., 2013; Liu et al., 2014].

Flavonoids are potent antioxidants and have aroused considerable interest recently because of their potential beneficial effects on human health in fighting diseases. The capacity of flavonoids to act as antioxidants depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. Quercetin, the most abundant dietary flavonol, is a potent antioxidant because it has all the right structural features for free radical scavenging activity. Therefore, the objective of present study is to determine the total flavonoid content of different extracts of *Ficus glomerata* (roots), Citrus aurantium (peels), Camellia sinensis (leaf), Spinacea oleracea (leaf) and Phaseolus vulgaris (seed) by using Aluminium Chloride colorimetric method (Patel et al., 2008; Chen et al., 1990).

MATERIALS AND METHODS

Plant Material

The plant materials were collected from Pune, Maharashtra, India. It was authenticated from botanical survey of India. The different parts of crude drugs were dried under shade and powdered well using a mixer.

Reference compounds and reagents

The standard Quercetin and other chemicals were procured from Research-Lab Fine Chem Industries, Mumbai, Maharashtra, India and analytical grade solvents were procured from Merck (Mumbai, India).

Preparation of herbal drugs extracts by using different extracting methods

1. Aqueous extraction by maceration method

In this method, the crude drugs were shade dried and coarsely powered. The aqueous extraction was carried out by maceration process with 10g of powdered drug using chloroform: water (1:99) for seven days, after completion of extraction the solvent was concentrated (Sithisarn, 2006, Wang et al., 2010).

2. Ethanolic extraction by microwave assisted method

In this method for 15g of crude drug, 50 ml 70% ethanol was used. The specified amount of material was mixed with calculated amount of solvent and kept in microwave [Catalyst Systems] for 5 minutes at % power 50 %, watt-350. The resultant mixture was filtered and then filtrate was concentrated (Sithisarn, 2006, Wang et al., 2010). The colour, consistency weight and the percent yield of the aqueous and ethanolic extract were documented.

Preliminary Phytochemical screening

The major secondary metabolites like flavonoids, alkaloids, phenolics, saponins, proteins, lipids/fats , tannins, amino acids, gums, mucilage and carbohydrates were screened with their respective tests from an aqueous and ethanol extracts of different species using various chemical tests [Harborne, 1973; Trease, 1989, Khandelwal, 2005].

Thin layer chromatography

The thin layer chromatography were performed where toluene: ethyl acetate: formic acid (9: 3: 1.75) as solvent system and silica gel 120 used as stationary phase.

Total flavonoid content

The Aluminium chloride colourimetric method was followed to determine total flavonoid content. In this method 1 mL of plant extract (1 mg/mL) and 2 mL of water were added in 10 ml volumetric flask. After 5 min the method was followed by the addition of 3 mL of 5 % sodium nitrite and 0.3 mL of 10 % aluminium chloride. After 6 min 2 mL of 1 M sodium hydroxide was added in the same volumetric flask and the volume adjusted to 10 mL with

water as solvent. Then an absorbance was measured at wavelength 510 nm. The percentage of total flavonoid content were calculated from calibration curve of quercetin (10-250µg) plotted by plotting graph absorbance vs concentration. The total flavonoids were expressed as quercetin equivalents in milligrams per gram sample (Chang et al., 2002).

RESULT

The extraction is the step in which separation of extract was carried out from plants by using organic solvents. There are different extractions techniques usually require more extraction time because of this there is risk of thermal degradation of thermolabile chemical compounds and to overcome this problem, there is need to find out another method. By considering this approach to get better efficiency, low solvent consumption and less extraction time microwave assisted extraction technique were studied [Chan et al., 2011].

In the present study extraction was followed by using microwave assisted extraction technique, Table 1 and Table 2 represents colour, consistency weight and the percent yield of the aqueous and ethanolic extract. The qualitative chemical analysis of aqueous and ethanolic extracts was given in Table 3. The thin layer chromatography data was given in Table 4.

Table 1: Represents colour, consistency weight and the percent yield of the aqueous extracts.

S. No.	Herbal drugs	Colour of extract	Consistency	Weight of extract (gm)	% Yield (W/W)
1.	*Ficus glomerata*	Brown	Semisolid	1.25	12.5
2.	*Citrus aurantium*	Brown	Semisolid	3.46	34.6
3.	*Camellia sinensis*	Greenish	Semisolid	2.28	22.8
4.	*Spinacea oleracea*	Greenish	Semisolid	2.93	29.3
5.	*Phaseolus vulgaris*	Brown	Semisolid	1.63	16.3

Table 2: Represents colour, consistency weight and the percent yield of the ethanolic extracts.

Sr No.	Herbal drugs	Colour of extract	Consistency	Weight of extract (gm)	% Yield (W/W)
1.	*Ficus glomerata*	Brown	Semisolid	0.10	1
2.	*Citrus aurantium*	Yellowish	Semisolid	2.35	23.5
3.	*Camellia sinensis*	Green	Semisolid	1.42	14.2
4.	*Spinacea oleracea*	Green	Semisolid	0.52	5.2
5.	*Phaseolus vulgaris*	Brown	Semisolid	0.66	6.6

Table 3: Qualitative chemical analysis for aqueous and ethanolic extracts.

Herbal drugs used	*Ficus glomerata*		*Citrus aurantium*		*Camellia sinensis*		*Spinacea oleracea*		*Phaseolus vulgaris*	
	AQ	ET	AQ	ET	AQ	ET	AQ	ET	AQ	ET
Flavonoids	+	+	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	-	-	+	+
Phenolics	+	+	+	+	+	+	+	+	+	+
Saponins	-	-	+	+	+	-	+	-	+	+
Proteins	+	+	+	+	+	+	+	+	-	+
Lipids/fats	-	-	-	-	-	-	-	-	+	+
Tannins	+	+	+	+	+	+	-	-	+	-
Amino acids	+	-	+	-	+	+	+	-	+	-
Gums and mucilage	-	-	-	-	-	-	-	-	-	-
Carbohydrates	+	+	+	+	+	+	+	+	+	+

AQ: Aqueous, ET: Ethanolic, Absent:-, Present: +.

Thin layer chromatography

The TLC for aqueous and ethanolic extracts of *Ficus glomerata*, *Citrus aurantium*, *Spinacea oleracea*, *Camellia sinensis* and *Phaseolus vulgari* was performed by taking Toluene: Ethyl acetate: Formic acid (9: 3: 1.75) as mobile phase solvent system and silica gel as stationary phase. For all the TLC plates, the R_f values were determined and given in Table 4.

Table 4: Rf value of aqueous and ethanolic exatrct.

Herbal drugs used	Aqueous extract R_f value	Ethanolic extract R_f value
Ficus glomerata	0.67	0.94
Citrus aurantium	0.5 0.31	0.72
Camellia sinensis	0.6	0.84
Spinacea oleracea	0.91	0.95
Phaseolus vulgaris	0.37	0.55 0.84

Total flavonoid content

The total flavonoid contents of the different extracts of *Ficus glomerata*, *Citrus aurantium*, *Spinacea oleracea*, *Camellia sinensis* and *Phaseolus vulgari* were calculated. It was observed that aqueous exatrct gives total flavonoids content 169, 52, 126, 520 and 262 mg/gm respectively while in the ethanol extract it was found to be 623, 102, 208, 458 and 456 mg/gm respectively. In this method the Quercetin was used as a standard and the results were expressed in Quercetin equivalent (mg/g). The standard calibration curve of Quercetin was given in figure 1.

Table 5: List of plants with total flavonoid content for aqueous and ethanolic extract.

Sr. No	Herbal drugs	Total Flavonoid content of aqueous extract (mg/gm)	Total Flavonoid content of ethanolic extract (mg/gm)
1.	*Ficus glomerata*	169	623
2.	*Citrus aurantium*	52	102
3.	*Camellia sinensis*	126	208
4.	*Spinacea oleracea*	520	458
5.	*Phaseolus vulgaris*	262	456

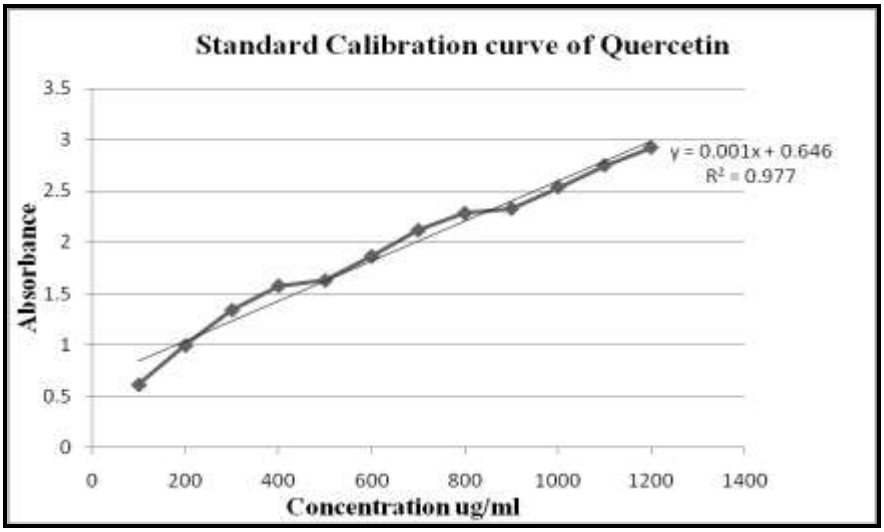

Figure 1: Standard calibration curve of Quercetin.

DISCUSSION

The TLC of both the extracts showed the spots which is the active chemical constituents. This suggested that water and alcohol were the appropriate solvent for extraction of flavonoids. Flavonoids are one of the most diverse and widespread group of natural compounds, are probably the most important natural phenolics. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties (Prasad et al., 2009; Kiranmai et al., 2011).

The Maceration method of extraction is simple and economical but time consuming is more (7 days) when compared to microwave synthesis extraction methods (Rafie et al., 2011). The Maceration extraction method gave the highest yield but lowest total flavonoid concentration (TFC), while the microwave synthesis gave a low yield but high total flavonoid concentration (accept for *Spinacea oleracea* in case of TFC), indicating that the active flavonoids in the herbal drugs were better extracted by 70% ethanol than water.

CONCLUSION

The results obtained in the present study indicate that aqueous and ethanolic extract of *Ficus glomerata* (root), *Citrus aurantium* (peel), *Camellia sinensis* (leaf), *Spinacea oleracea* (leaf) and *Phaseolus vulgaris* (seed) contain good amount of flavonoid content and can be used as a natural source of antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases.

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