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(RESEARCH ARTICLE)

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Quantitative analysis of quercetin in various extracts of *Curcuma amada* by high performance liquid chromatography

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Abstract

Herbal medicine and their preparations have been widely used from the thousands of years in developing and developed countries in the primary health care of society and community. Quality control is one of the very important and essential steps in the manufacturing of herbal preparations as quality of product affects the safety and efficacy of medicines. Quality control is mainly applied for both raw materials along with excipients used and finished product. Flavonoids are the class of polyphenolic compounds, which are mainly distributed throughout the plant kingdom. Quercetin is a flavonoid which shows major pharmacological activities like anticancer, hepatoprotective activity, anti-spasmodic, and anti-inflammatory activity. In present investigation qualitatively and quantitatively estimation of the extracts of *Curcuma amada* has been carried out.

Keywords: Herbal medicine; Qualitative; Quantitative; Quercetin; Bioactive; HPLC

1. Introduction

People on all continents have used hundreds to thousands of indigenous plants for treatment of ailments since prehistoric times. Many plants synthesize substances that are useful to the maintenance of health in humans and other animals. These include aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins [1].

According to an estimate of the World Health Organization (WHO), about 80% ofthe world population still uses herbs and other traditional medicines for their primary health care needs. Herbal medicine products are dietary supplements that people take to improve their health and are sold as tablets, capsules, powders, teas, extracts and fresh or dried plants. Herbals are traditionally considered harmless and increasingly being consumed by people without prescription [2]. Hence every single herb needs to be quality checked to ascertain that it confirms to quality requirement and delivers the properties consistently. Standardization assures that products are reliable in terms of quality, efficacy, performance and safety [3].

Over the past decades, HPLC has received the most extensive application in the analysis of herbal medicines. Reversed phase (RP) columns may be the most popular columns used in the analytical separation of herbal medicines. Preparative and analytical HPLC are widely used in pharmaceutical industry for isolating and purification of herbal compounds. There are basically two types of preparative HPLC: low pressure HPLC (typically under 5 bar) and high-pressure HPLC (pressure >20 bar). The important parameters to be considered are resolution, sensitivity and fast analysis time in analytical HPLC whereas both the degree of solute purity as well as the amount of compound that can be produced per unit time i.e. through put or recovery in preparative HPLC.

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2. Material and methods

2.1. Collection of Plant material

The rhizome of *Curcuma amada* was collected from rural area of Bhopal(M.P), India in the months of January, 2021.

2.2. Authentication of plant

The herb was authenticated by a senior Botanist Dr. Jasvider Mehta, Career College, Bhopal (M.P.).

2.3. Selection of plants for the study

Ethnobotanical surveys were conducted in different tribal localities of Madhya Pradesh. The technique taken for information collection was to interview tribes, local medicine men and discuss the therapeutic use of local crops in the therapy of multiple illnesses.

2.4. Preparation of plant material for study

Plant material (rhizomes) selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time. Then the plant material was shade dried without any contamination for about 3 to 4 weeks. Dried plant material was grinded using electronic grinder. Powdered plant material was observed for their colour, odour, taste and texture. Dried plant material was packed in air tight container till any further use.

2.5. Extraction Procedure

Following the procedure for preparing the consecutive of dried and powdered herbs from the shade was introduced:

2.5.1. Defatting of plant material

Curcuma amada was washed at room temperature with powdered plant material (rhizomes). The dried floral shade was heavily powdered and subjected to extraction in a soxhlet apparatus with petroleum ether (60-80 °C). The extraction proceededuntil the material had been defatted

2.5.2. Extraction by hot continuous percolation process

80 g of *Curcuma amada* dried rhizomes were successive extracted using various solvent (chloroform, ethyl acetate, ethanol, and aqueous) and using different drug: Solvent ratios for distinct times using warm constant percolation [4]. Over their boiling points, the contents were evaporated. Finally, the percentage yields of the dried extracts were calculated.

2.6. Determination of physio-chemical Parameters

2.6.1. Determination of Ash Values

Determination of Total Ash: 2 g of accurately weighed powder was incinerated in a tarred platinum or silica dish at a temperature not exceeding 450 ºC until free from carbon, cooled and weighed [5]. If a carbon-free ash could not be obtained in this way, the charred mass was exhausted with hot water, the residue was collected on an ashless filter paper, incinerated, evaporated to dryness, and ignited at a temperature not exceeding 450 °C. The ash thus acquired was then cooled, weighed, and with regard to the air-dried drug, the proportion of ash was calculated.

2.6.2. Determination of Acid Insoluble Ash

The ash obtained from the above operation was boiled with 25 ml of diluted hydrochloric acid for 5 minutes and the insoluble matter was gathered either in a gooch crucible or on an ashless filter paper. The resulting insoluble matter was washed with warm water and filter paper was rubbed together with filter paper to a constant weight. Based on the air-dried drug, the proportion of acid-insoluble ash was calculated.

2.6.3. Determination of Water Soluble Ash

The ash was cooked with 25 ml of water for 5 minutes, the insoluble material collected in a Gooch crucible, or on an ashless filter paper, washed with hot water and ignited at a temperature not exceeding 450 °C for 15 minutes. The

insoluble matter's weight was subtracted from the ash's weight. Water soluble ash was the distinction in weight. Referring to air-dried drug, the proportion of water-soluble ash was calculated.

2.6.4. Determination of Extractive Value

Determination of Water Soluble Extractive

5 g of air-dried powder was macerated for 24 hours in a closed flask with 100 ml of chloroform water of the specified strength, shaking at an interval of six hours. It was then eighteen hours permitted to stand. The macerate was quickly filtered to avoid solvent loss. In a tarred flat bottom shallow dish, 25 ml of the filtrate was evaporatedto dryness and dried at 105°C to a constant weight and lastly weighed. Referring tothe air-dried drug, the proportion of water-soluble extract was calculated.

2.6.5. Determination of Moisture Content (loss on drying)

Approximately 10 g of rhizomes (without preliminary drying and cutting in areas approximately 3 mm thick) was put in a tarred evaporation pot after accurate weighing [6]. It was then washed and weighed at 105°C for 5 hours. Drying was continued and the rhizomes were weighed at 1 h interval until the difference between two successive weighing corresponded to not more than 0.25 percent. Constant weight was achieved when two consecutive weights showed no more than 0.01 g difference after drying for 30 minutes and cooling in a desiccator for 30 minutes.

2.7. Quantitative analysis

Quantitative analysis is an important tool for the determination of quantity of phytoconstituents present in plant extracts. For this TPC and TFC are determined.ethanolic extract obtained from rhizomes of *Curcuma amada* plant material of subjected to estimate the presence of TPC and TFC by standard procedure.

2.7.1. Total flavonoids content estimation (TFC)

Determination of total flavonoids content was based on aluminium chloride method [7].

Preparation of standard: 50 mg quercetin was dissolved in 50 ml methanol, and various aliquots of 25- 125 µg/ml were prepared in methanol.

Preparation of extract: 10 mg of dried extracts dissolved in 10 ml methanol and filter. One ml (1 mg/ml) of this extract was for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 1 ml of extract or standard and allowed to stand for 60 min at room temperature; absorbance was measured at 420 nm.

2.7.2. Determination of total alkaloid content (TAC)

The extract of the plant (20 mg) was dissolved and filtered in 1ml of 2 N HCl. This solution was transmitted to a separate funnel, adding 5 ml of green bromocresol and5 ml of phosphate buffer. The mixture was shaken by vigorous shaking with 1, 2, 3and 4 ml chloroform and gathered in a volumetric flask of 10 ml and diluted with chloroform to the quantity. A set of conventional atropine reference alternatives (40, 60, 80, 100 and 120 µg / ml) was prepared in the same way as previously outlined.An UV / Visible spectrophotometer was used to determine the absorbance for test and normal solutions against the reagent blank at 470 nm. The total content of alkaloids was expressed as mg of extract AE/100 mg.

2.8. Identification of marker compound (Quercetin) by HPLC

2.8.1. Reagents and chemicals

Quercetin was kindly provided by Scan Research Laboratories, Bhopal (India). Methanol and acetonitrile were of HPLC grade and purchased from Merck Ltd, New Delhi, India. Water used was of HPLC grade water from Merck Ltd, New Delhi, India.

2.8.2. Instrumentation

A Labindia 3000 thermospectronic model + 1 cm UV / VIS spectrophotometer. Appropriate quartz cells were used to determine λ_{max}. The HPLC (Waters) system consisted of a U.V. pump. Visible detector, column of Thermo C18 (250 X 4.6 mm, 5µm), software of Data Ace.

2.9. Chromatographic conditions

The chromatographic assessment was conducted at ambient temperature on an analytical column RP-C18 with a mobile phase consisting of acetonitrile: methanol (50:50 v / v) and isocratically eluted at a flow rate of 1 mL min-1. For each sample run, a small sample volume of 20 µL was injected into the HPLC system. The chromatogram was tracked at a wavelength of 256 nm using UV detection [8].

2.9.1. Preparation of standard stock solution

10 mg of quercetin was correctly measured and transferred to a volumetric flask of 10ml, and the amount was adapted to the methanol mark to provide a 1000 ppmstock solution.

2.9.2. Preparation of working standard solution

Up to 10 ml of Quercetin stock solutions were taken and diluted. From this solution0.5, 1.0, 1.5, 2.0, 2.5 ml solutions have been transmitted to 10 ml volumetric flasks and constitute a quantity of up to 10 ml with portable stage, providing normal medication solution concentrations of 5, 10, 15, 20, 25 µg / ml.

2.9.3. Analysis of extracts

10 mg sample was carried in 10 ml volumetric flask and diluted with methanol up to the mark; the resulting solution was filtered through Whatmann filter paper and lastly the volume was produced up to 1000 µg/ml marked with the same solvent. Using Whatmann filter paper No. 41, the resulting solution was filtered again and then sonicated for 10 min.

3. Results

Table 1 Organoleptic property of quercetin

Color	**Yellow powder**
Odor	Odorless

Table 2 Major peaks observed in the FTIR spectrum

Bands	**Wave number(cm-1)**
C-O Stretching	1133.75
O-H Stretching	3577.93
C=C stretching vibration	1729.64
C=C stretching vibration	1681.66

Table 3 Solubility studies of quercetin in different solvent

S. No.	**Solvent Used**	**Solubility of quercetin**
1.	Water	Insoluble
2.	0.1 N HCl	Soluble
3.	Ethanol	Freely Soluble
4.	Methanol	Freely Soluble
5.	Chloroform	Insoluble
6.	0.1 N NaOH	Soluble
7.	7.4 pH Phosphate Buffer	Soluble

Table 4 Results of percentage loss

S. No.	Plant name	Description	Weight in gms.	% loss
1.	*Curcuma amada*	Weight of plant material in wet, fresh condition	120	
2.		Weight of plant material after drying at room temperature	80	
3.		Loss in weight on drying	120-80=40	33%

Table 5 Physicochemical test of *Curcuma amada*

S. No.	Parameters	Value in w/w%
1.	Total ash	3.96
2.	Water soluble ash	6.75
3.	Acid insoluble ash	5.20
4.	Loss on drying	2.56

Table 6 Determination of percentage yield of different extract of *Curcuma amada*

Extract	Percentage yield			
	Chloroform	Ethyl Acetate	Ethanolic	Aqueous
Curcuma amada	0.58	0.79	2.53	2.10

Table 7 Preparation of calibration curve of Quercetin

Standard Concentration µg/ml	Absorbance						Mean
	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	Rep-6	
0	0	0	0	0	0	0	0
25	0.265	0.264	0.265	0.264	0.265	0.265	0.265
50	0.532	0.531	0.532	0.531	0.532	0.531	0.532
75	0.765	0.764	0.765	0.764	0.765	0.764	0.765
100	1.013	1.012	1.013	1.014	1.013	1.013	1.013
125	1.195	1.195	1.196	1.195	1.194	1.195	1.195
Correlation Coefficient(r^2)							0.009
Slope (m)							0.024
Intercept (c)							0.996

Table 8 Preparation of calibration curve of Atropine

Standard Concentration µg/ml	**Absorbance**						**Mean**
	Rep-1	**Rep-2**	**Rep-3**	**Rep-4**	**Rep-5**	**Rep-6**	
0	0	0	0	0	0	0	0
40	0.335	0.334	0.336	0.335	0.338	0.337	0.336
60	0.498	0.496	0.497	0.496	0.498	0.499	0.497
80	0.661	0.662	0.661	0.662	0.664	0.663	0.662
100	0.836	0.836	0.837	0.838	0.837	0.836	0.837
120	1.023	1.023	1.024	1.025	1.024	1.024	1.024
Correlation Coefficient(r^2)							0.008
Slope (m)							0.005
Intercept (c)							0.999

Table 9 Total flavonoids and alkaloid content of *Curcuma amada*

Estimation	***Curcuma amada***		
	Ethyl acetate	**Ethanolic**	**Aqueous**
Total Flavonoid (mg/100mg)	0.423	0.965	0.758
Total alkaloid (mg/100mg)	-	0.536	-

Table 10 Preparation of Calibration curve of Quercetin

S. No.	**Concentration (µg/ml)**	**Mean AUC**
1.	0	0
2.	5	342.45
3.	10	618.66
4.	15	898.85
5.	20	1202.75
6.	25	1466.24

Table 11 Characteristics of the analytical method derived from the standardcalibration curve

Compound	**Linearity range µg/ml**	**Correlation co-efficient**	**Slope**	**Intercept**
Quercetin	5-25	0.998	58.24	26.80

Table 12 Quantitative estimation of Quercetin in extract of *Curcuma amada*

S. No.	***Curcuma amada***	**RT**	**% Assay**
1.	Standard	2.621	
2.	Ethanolic extract	2.637	0.763

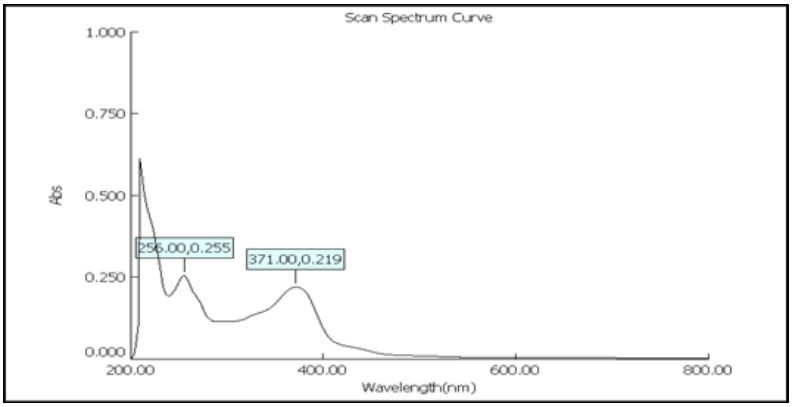

Figure 1 Wavelength maxima of quercetin in phosphate buffer pH 7.4

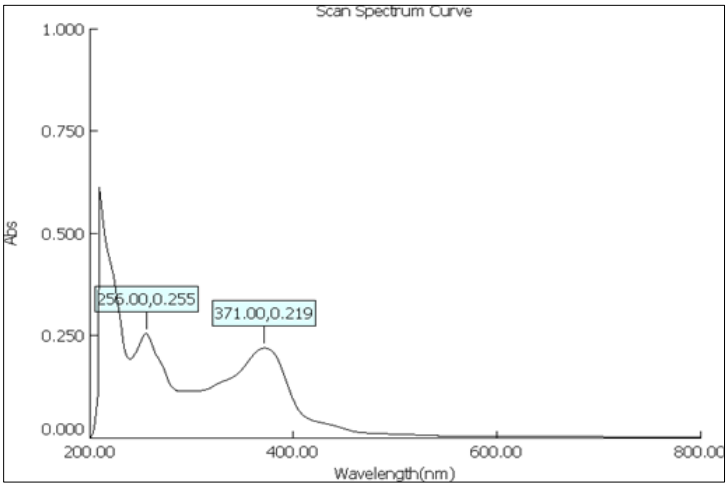

Figure 2 Wavelength maxima of quercetin in 0.1 N HCl

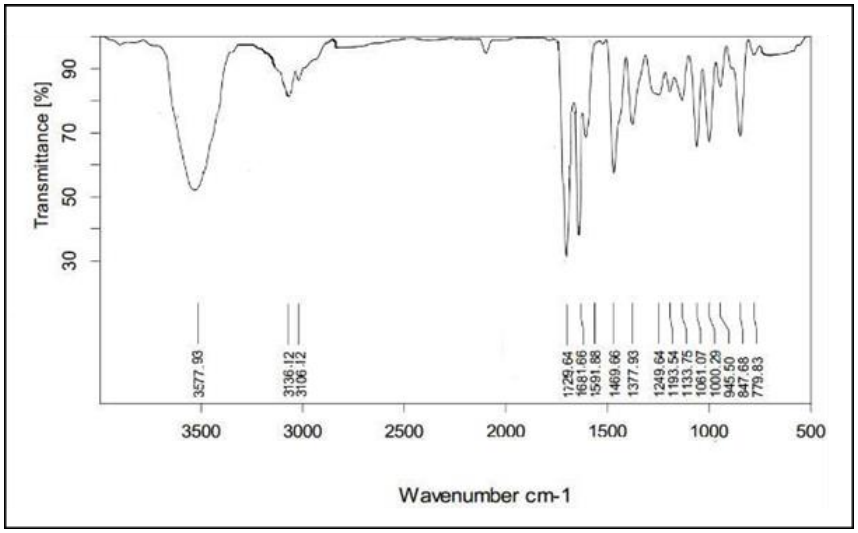

Figure 3 FT-IR spectra of Quercetin

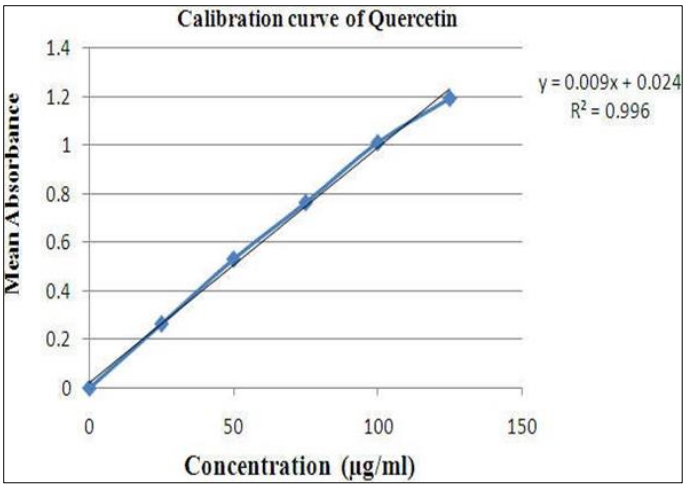

Figure 4 Calibration curve of Quercetin

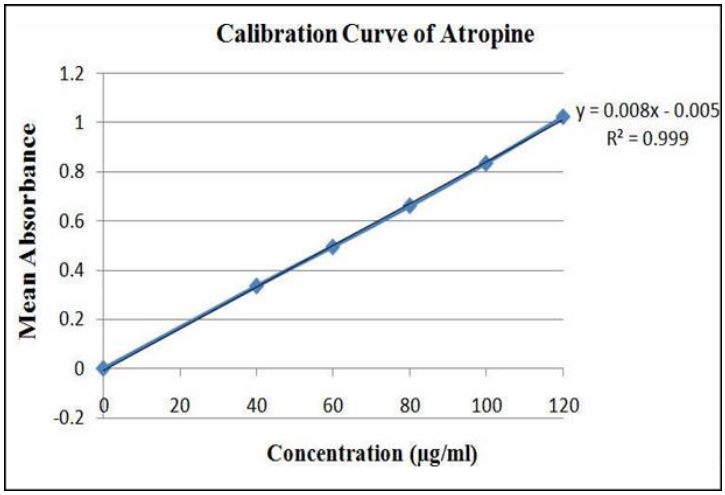

Figure 5 Graph of calibration curve of Atropine

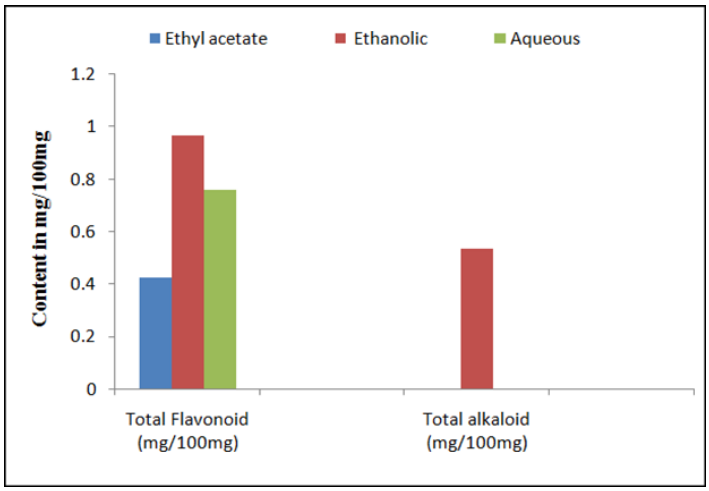

Figure 6 Total flavonoids and Alkaloid content of *Curcuma amada*

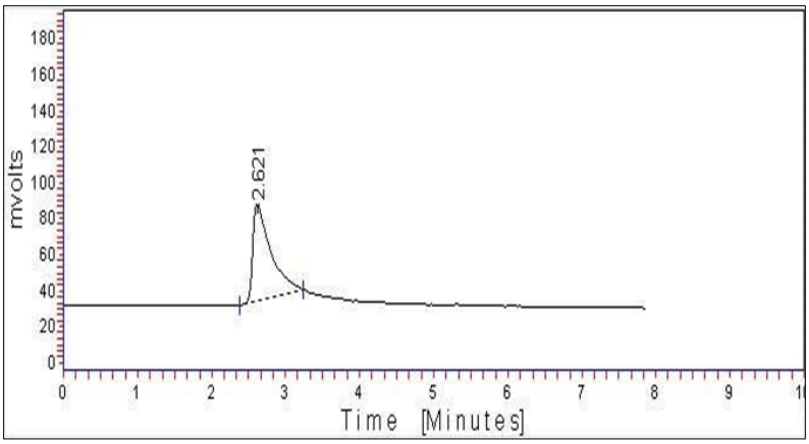

Figure 7 Chromatogram of standard Quercetin

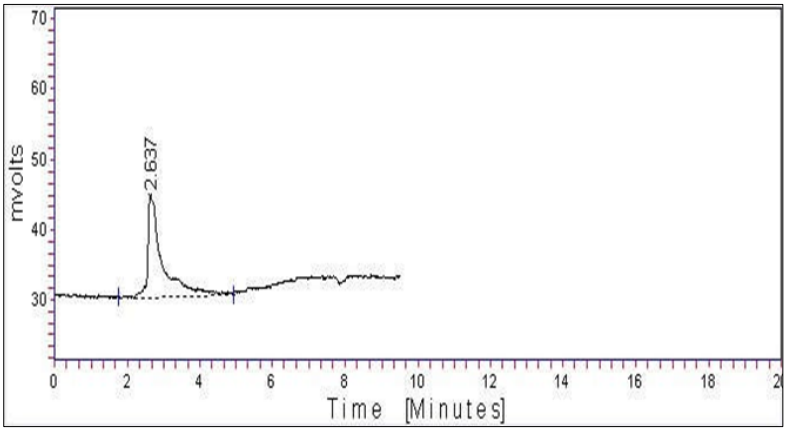

Figure 8 Chromatogram of ethanolic extract of *Curcuma amada*

4. Discussion

Identification and characterization of quercetin was performed, quercetin was found to be yellow and odorless powder. The wavelength maximum for quercetin in phosphate buffer pH 7.4 was found 256nm which compliance with standard. The spectrum of drug was authenticated by FTIR spectroscopy. The presence of characteristic peaks associated with specific structural characteristics of the drug molecule was noted. The appearance or disappearance of peaks and/or the shift of their positions are often indications of interactions such as hydrogen bonding. Quercetin presented characteristic peak at 3577.93 cm^{-1} due to OH stretching, 1133.75cm^{-1} was due to CO stretching vibration. At 1729.64cm^{-1} presenting C=C stretching vibration.

When the data obtained from FTIR spectra is compared with the standard spectra studied it was observed that there are similar peaks for functional groups in quercetin, this shows the purity of quercetin.

It was found that quercetin was practically insoluble in water and chloroform, freely soluble in ethanol and methanol, soluble in 0.1 N NaOH, 0.1 N HCl and 7.4 pH phosphate buffer. The percentage of loss on drying of quercetin was found 0.68±0.013% w/w.

Table no. 6 showed the percentage yield of different extract of roots of *Curcuma amada* exhibited higher yield in ethanolic extract 2.53%. In petroleum ether, ethyl acetate, ethanolic and aqueous extract the percentage yield was found 0.58%, 0.79%, 2.53% and 2.10% respectively.

Phenolic group are a class of antioxidant component which act as free radical terminators and their activities may be related to their abilities to chelate metals, inhibit lipoxygenase and scavenge free radicals. The amount of total phenol

wasestimated with the Folin-Ciocalteu reagent. The content of total phenolic compounds (TPC) was expressed as mg/100mg of Gallic acid and total phenol (mg/100mg) was found to be 0.423, 0.965 and 0.758mg/100mg for ethyl acetate and ethanolic and aqueous extract respectively.

Alkaloid as one of the most diverse and wide spread group of compounds is mainly the most significant natural phytoconstituent. Total alkaloid content was calculated as atropine equivalent and total alkaloid (mg/100mg) was found to be 0.536 mg/100mg respectively.

A reverse phase C-18 column equilibrated with mobile phase methanol: acetonitrile (50:50, v/v) was used. Mobile phase was filtered through Whatmann filter paper and degassed. Mobile phase flow rate was maintained at 1 ml/min and effluents were monitored at 256 nm. The sample was injected using a 20 µl fixed loop, and the total run time was 10 min. The sample solution was chromatographed and a concentrationof quercetin in extract sample was found out using regression equation. The percentage of quercetin was found 0.763% in ethanolic extract.

5. Conclusion

Herbal medicine and their preparations have been widely used from the thousands of years in developing and developed countries in the primary health care of society and community. Quality control is one of the very important and essential steps inthe manufacturing of herbal preparations as quality of product affects the safety and efficacy of medicines. Quality control is mainly applied for both raw materials along with excipients used and finished product. Flavonoids are the class of polyphenolic compounds, which are mainly distributed throughout the plant kingdom. Quercetinis a flavonoid which shows major pharmacological activities like anticancer, hepatoprotective activity, anti-spasmodic, and anti-inflammatory activity. In present investigation quantitative estimation of extracts of *Curcuma amada* has been done.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors (Mrs. Neha Rajput, Dr. Sarita Karole*, Dr. Anup K Chakraborty, Dr. Kavita R. Loksh) declare no conflict of interest.

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