International Journal of Scientific Research in Engineering and Management (IJSREM)
Volume: 07 Issue: 07 | July - 2023 SJIF Rating: 8.176 ISSN: 2582-3930

Pharmacognostical and Phytochemical Analysis of *Curcuma longa* Leaves Extract with its Antimicrobial Activity

Vaishali Dange, Pratiksha Wani
Institute of Biosciences and Technology, M.G.M University, Aurangabad Maharashtra
E-mail: vdange@mgmu.ac.in,
pratiksha.ashok.wani@gmail.com

ABSTRACT:

Context:

Aims: The purpose is to study Pharmacognostical characteristics, Physicochemical evaluation, Phytochemical study and Antimicrobial assay of *Curcuma longa* leaves extract.

Methods and Material: Different qualitative tests were performed to confirmed the presence of phytochemical compounds under consideration followed by quantitative analysis using sophisticated techniques such as spectrophotometric, Thin layer chromatography (TLC) and column chromatography.

Results: In pharmacognostic study the moisture content of *Curcuma longa* leaves is 1.75%, water and alcohol soluble extractive value is 19.25 and 12.6 % respectively, total ash obtain is 3.35%. Acid insoluble and water-soluble ash value is 1 and 1.2% respectively. phytochemical analysis of *Curcuma longa* was carried out by using various solvent (methanol, acetone and aqueous) extract crude sample for presence of secondary metabolites. The qualitative phytochemical analysis showed the presence of cumarin, fatty acids, terpenoids, tannin, phenolic compounds, flavonoids, reducing sugar and glycosides in respective solvent extract. The quantitative estimation of total phenolic content of *Curcuma longa* leaf extract for methanol, acetone and aqueous are (1.52,1.22 and 0.46). and for the flavonoid content of *Curcuma longa* leaf extract for methanol, acetone and aqueous are (1.46,1.34 and 0.78). the antimicrobial activity of *Curcuma longa* leaves extract against human pathogenic microorganisms (*E. coli and B. thurengenesis*). And one species of fungus (*A. niger*) by paper diffusion assay method performed on nutrient agar and Potato dextrose agar respectively. In antimicrobial analysis methanol and acetone extract was effective against *E. coli* and *B. thurengenesis.* The inhibitory zone was found to be ranging from 8mm to 20mm in diameter.

Conclusions: This study may be useful a source of information concerned to various field such as pharmacology, ethnology, phytochemistry and industrial microbiology for future aspect.

KEYWORDS: Quercetin, *Curcuma longa* leaves extract, Thin layer chromatography, Column chromatography, Spectrophotometry, antimicrobial assay, Phytochemical screening, Pharmacognostic study

INTRODUCTION

In ancient time people search for drugs in nature for the rescue of their diseases and their other health problems. For this preliminary they depends on the plants for the treatment of their diseases in place of animals. The oldest written medicinal document of plants has been reported in the Sumerian as well as Babylon's civilization in the earth. The utilization of herbs was the very a good practice in the Indian culture at the times of the rishi-munis. The Ayurveda system of the medicinal plants was a very old literature which is still very reliable and authentic source of information of the medicinal plant. [1]

India become the world's largest manufacturer of turmeric as it produces tones of turmeric plants every year. The rhizomes of turmeric are mostly considered as the useful part of the plant and leaves was not given care but now Indian and Malaysian cookery are used the turmeric leaves as both fresh and dried extracted form, as a basic ingredient in curry powders and are purported to improve digestion and reduce gas and bloating. *Curcuma longa* (Haridra), *Curcuma zedoria* (Zedoary), *Curcuma aromatica* (Vana Haridra), *Curcuma caesia* (Kali Haridra), *Curcuma angustifolia*, and *Curcuma amada* (Amaragandhi Haridra) are species of *Curcuma* which are traditionally used for their antifungal, antibacterial, wound healing, anticancer, antioxidant, and anti-inflammatory activity respectively.[2]

Plant are rich in a wide variety of secondary metabolites such as tannins, phenols, terpenoids, alkaloids and flavonoids which have been proved invitro to have antimicrobial properties. Out of all the secondary metabolites phenol are the major group metabolites comprising of both medicinal and nutritional properties.[3] Flavonoids, another class of secondary metabolites having a benzo-y-pyrone ring consider under the polyphenolic group. These compounds possess greater biological activity solely depends on its chemical nature, degree of hydroxylation, polymerization, substitution or conjugation.[4]

As this plant has considerable therapeutic potential, the extraction and characterization of the essential bioactive compounds with vital medicinal properties may provide opportunities relating to pharmaceutical applications. Therefore, in this review, we have compiled and critically analysed the reported studies on the phytochemical and pharmacological properties of *C. longa* leaves we hope this will provide future insight into the medical application of *C. longa* for the treatment of various diseases.

MATERIALS AND METHODS

Materials

Collection of samples

The leaves sample were collected from the lord's herbal nursery and medical research Aurangabad, such as *Curcuma longa* leaves.

Pharmacognostical study

Fresh leaves were sun dried and pulverized in a mechanical grinder to obtain course powder and passed through sieve, stored in airtight amber coloured bottle and used for future present work [5]

Macroscopical analysis

Macroscopical examination of the plant was done by the observation of morphological characters such as size, shape, margin, base surface, colour, odour, and taste of *Curcuma longa* leaves [7].

Microscopical analysis

The *Curcuma longa* leaf were first cleaned with distilled water. Then take a thin section of leaf and then transfer in HCl containing watch glass. After 4-5 min this thin section transfer to glass slide add a drop of glycerol to this slide put the coverslip and observe under the microscope 10X,40X. [6-7]

Physiochemical evaluation

Physical parameters such as moisture content, moisture content ash values, acid insoluble ash, water soluble ash were determined as per procedures mentioned in accordance with WHO guidelines [8-10]

Determination of moisture content

About 42.408g of the leaf was weighed and dried in an oven at $100^0 – 105^0$ C. it was cooled in a desiccator and again weighed. The loss on drying was calculated with the reference to the amount of the dried powder taken and results obtained.

$$\text{Moisture content (\%)} = \frac{\text{Initial weight} - \text{Oven dry weight}}{\text{Oven dry weight}} \times 100$$

Determination of extractive values

Determination of water soluble extractive value

4g of dried leaf powder was socked in 100ml of water for 1 hr. and mixed properly. The mixture was boiled 100^{0}C on water bath and then filtered. Filtrate was evaporated in pre-weighed porcelain dish and dried at 105^{0}C. water soluble extractive value was calculated.

$$\text{Water soluble extractive value (\%)} = \frac{\text{Weight of residue}}{\text{Weight of drug}} \times 100$$

Determination of alcohol soluble extractive value

1g dried leaf powder material was macerated with 25 ml of alcohol in shaking condition and allowed to stand for 16 hr. and filtered. Filtrate was than evaporated in pre-weighed porcelain dish and dried at 105^{0}C. Alcohol soluble extractive value was calculated. (alcohol soluble extractive value was calculated same as above)

Determination of ash values

Ash values like total ash, acid insoluble ash of leaf of *Curcuma longa* was determine by following methods.

Determination of total ash

1g leaf powder was taken in pre weighed silica crucible and incinerated in a muffle furnace at 500^0-600^{0}C.till carbon free ash was obtained. Percentage of ash was calculated with reference to initial weight of dried powder.

$$\text{Total ash (\%)} = \frac{\text{Weight of ash obtain}}{\text{Weight of crude drug}} \times 100$$

Determination of acid insoluble ash

Ash obtained from total ash was boiled for 5 min with 12.5ml of 1N HCl and filtered using ashless filtered paper to collect insoluble matter. The filter paper was transferred into a pre weighed silica crucible and incinerated at 300^{0}C in hot air oven until free from carbon. Percentage of acid insoluble ash was calculated with the reference to dried leaf powder.

$$\text{Acid insoluble ash (\%)} = \frac{\text{Weight of acid insoluble ash}}{\text{Weight of crude drug}} \times 100$$

Determination of water soluble ash

Ash obtain from total ash was boiled for 5 min with 12.5 ml of water and filtered using ashless filter paper to collect insoluble matter. The filter paper was transferred into a pre weighed silica crucible and incinerated at 450^{0}C in hot air oven until free from carbon. Percentage of acid insoluble ash was calculated with the reference to dried leaf powder. (water soluble ash value was calculated as same above)

Phytochemical screening

For the phytochemical analysis of *Curcuma longa* leaves extract prepare by using 3 different solvents such as methanol, acetone, aqueous. [11]

Preparation of leaf extraction

10g powdered leaves were successively extracted with 100ml methanol, acetone and aqueous using Soxhlet apparatus in a thimble and extraction was carried out for 3 hrs. at 70^{0}C. the extract was filtered and stored in air tight container at -20^{0}C for a further phytochemical analysis.

Qualitative phytochemical screening

Qualitative phytochemical tests were carried out for carbohydrates, proteins and amino acids, alkaloids, glycosides, flavonoids, tannins, phenolics, steroids, anthraquinone, saponin, terpenoids, and phlobatannins according to standard method by Khandelwal and Kokate. [11]

Quantitative phytochemical screening

Quantitative phytochemical screening was caried out for phenols and flavonoids. [12]

Total phenolic content determination

The phenolic compound content was determined by the Folin-Ciocalteu method [13]. To measure total phenolic content 0.5 ml of the turmeric leaf extract was mixed with 0.3ml of distilled water. Then 0.75ml of 10% sodium carbonate solution was added and incubate for 3min. after that, 0.9 ml distilled water and 0.25 ml of Folin- Ciocalteu reagent (Sigma Chemical, St. Louis, MO) were added to the mixture and incubated for another 30min at room temperature. Absorbance was measured at a wavelength of 750nm. The result was expressed as mg of gallic acid (mg GAE/g) based on the gallic acid standard curve.

Total flavonoid content determination

Total flavonoid content was determined by the aluminium chloride colorimetric method [13-14]. To measure the total flavonoid content 0.5 ml of the turmeric leaf extract was mixed with 1.5ml of methanol, 2.8 ml of distilled water, and 0.3 ml of 3% sodium nitrite. After reaction, 0.1 ml of 10% aluminium chloride and 0.1ml pf 1M potassium acetate were added and incubated for 30min at room temperature. Then, absorbance was measure at a wavelength of 415nm. The result was expressed as mg of quercetin (mg QCE/g) based on the quercetin standard curve.

Chromatographic study

Thin layer chromatography

Preparation of thin layer chromatography

The adsorbent (silica gel G) slurry was prepared in water in the ratio of (1:2). The glass plates (20cm x 5cm) were cleaned. The slurry was poured into a glass slide and spread with the spreader. These plates were air dried and activated in hot air oven at 105^0C for 30 min. The plates were used as the stationary phase or pre-coated aluminium plates coated with silica gel G F254 (merk) were also used for analysis. [15-17]

Sample preparation

The extracts were dissolved in methanol and acetone and the spot was applied on the TLC plates using capillary tube.

Development of chromatogram

After drying of the spot, the plates were developed in a chromatographic tank containing the solvent system. After one third of the plate was developed the plates were taken outside and dried. The TLC plate was examined visually of under UV light.

Solvent system

Silica gel G act as Stationary phase. Whereas different mobile has been prepared viz. n- butanol: acetic acid: distilled water (2:2:6), chloroform: methanol (7:3) and ethyl acetate: water: butanol (2.5:1.5:0.5)

The best result is obtained by n-butanol: acetic acid: distilled water and this solvent system is used for the column chromatography. Visual / UV light and 1% methanolic spray are act as a Detecting agent.

The RF values were calculates using the formula [distance travelled by solute / distance travelled by solvent].

Column chromatography

Preparation of silica slurry

Weighed the 10 gm of silica powder and mix with the solvent mobile phase which used for column preparation (n-butanol: acetic acid: distilled water). Incubate the slurry for 1-2 hrs. at room temperature. [18]

Column preparation

A suitable size long cylindrical column should be stand firm on a chromatography stand. Firstly, add the glass beads in the column then add the slurry up to 4-5 cm long. Then add the glass beads again and load the sample about 400-500ul.After that fractions were collected about 1-2 ml of methanol and acetone solvent leaf extract separately. The acetone leaf extract samples 45 fractions were collected and take a OD of these all fractions at 360nm and plot the graph and run the TLC plate for the highest OD obtained fraction. Same procedure for the methanol leaf extract sample 14 fractions were collected and take a OD of these all fractions at 360nm and plot the graph and run the TLC plate for the highest OD obtained fraction.

Antimicrobial assay

Preparation of active bacterial cultures

For the preparation of active culture of microbial isolates: 100ml of nutrient broth media was prepared and autoclaved. In laminar air flow it as distributed in screw cap bottles approximately 20ml in each bottle and loopful culture of (*E.coli, B.thurengenesis*) inoculated in respective screw cap bottles are stored at 40^0C for further use. [19].

Preparation of active fungal cultures

For preparation of active fungal culture isolates 100ml of potato dextrose broth [PDB] was prepared and was autoclaved. In laminar air flow it was distributed in screw cap bottles approximately 20ml in each bottle and incubated at 37^0C for further use.

Preparation of nutrient agar and PDA plates for disc assay

Plain plate of Nutrient agar and Potato dextrose agar media were prepared. After that plates were spread with the *E.coli*, *B. thurengenesis*, and *A. niger*. After that methanol, acetone and aqueous extract containing disc were put on this plate and pates were incubated at 37^0C for 48hr. the zone of inhibition was observed.

RESULTS AND DISCUSSION

Pharmacognostical study

Plant was authenticated by detailed observation of macroscopical and microscopical characters.

Morphological studies:

leaves of *Curcuma longa* are greenish- yellow in colour, containing characteristics odour, bitter test, smooth, ovate, acute entire, and 31 x 9.5 cm in size along with petiole of 6.1 cm long.(Table 1) (fig.1)

Characters	Parameters
Colour	Greenish yellow
Taste	Bitter
Surface	Smooth
Elevation	Ovate
Margin	Entire
Size	31 x 9.5cm

Table 1. Morphological characters of *Curcuma longa* leave

Fig 1: *Curcuma longa* leaf

Microscopic studies

Anatomy of leaf : the leaves of Curcuma longa are long and dorsiventral with distinct midrib from the lamina. The single layered polygonal epidermal cells are present which containing cuticle on the outer layer along with unicellular covering trichome. Moreover, and wide band of phloem are present. The lamina is uniformly flat and petiole is elliptical with even and smooth surface.(Fig.2)

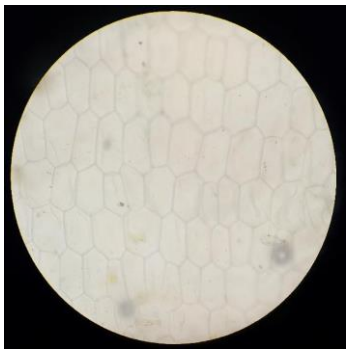


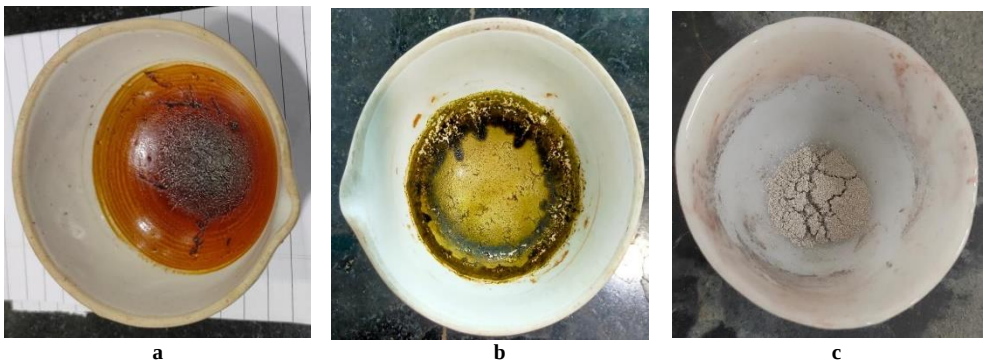
Fig 2: Polygonal cells of *Curcuma longa* leaf

Physicochemical evaluation

The physicochemical evaluation of Curcuma longa leaf powder carried out for different parameters such as moisture content, water soluble extractive value, alcohol soluble extractive value, total ash count, acid insoluble ash value, water soluble ash value, the proximate analysis of Curcuma longa leaf powder obtained results presented in Table no. 2 (Fig.3).

Parameters	Value (%)
Moisture content	1.57
Water soluble extractive value	19.25
Alcohol soluble extractive value	12.6
Total ash	3.35
Acid insoluble ash	1
Water insoluble ash	1.2

Table no.2 Physicochemical parameters of *Curcuma longa* leaf powder



a **b** **c**

Fig 3: powder study. (a) water soluble extract (b) alcohol soluble extract (c) total ash

Phytochemical screening:

1. **Qualitative phytochemical screening**

The phytochemical tests were conducted to identify the components of bioactive compounds contained in *Curcuma longa* leaf extract. Components of bioactive compounds tested in this study were: alkaloid, tannin, anthocyanin, coumarin, emodins, proteins, flavonoids, diterpenes, phytosterol, phenol, phlobatannin, leucoanthocyanin, anthroquinone, cardiac glycoside and carbohydrate test. The phytochemical test of *Curcuma longa* leaf extract using methanol, acetone, and aqueous as shown in Table 3**.**

Sr. no.	Solvents → Parameters ↓	Methanol	Acetone	Aqueous
1	**Alkaloid**			
	a) Wagner's test	-	-	-
2	**Tannin**	-	+	+
3	**Anthocyanin**	-	-	-
4	**Coumarins**	-	+	+
5	**Emodins**	-	-	+
6	**Proteins**	-	-	-
7	**Flavonoids**			
	a) Alkaline reagent test	+	+	+
8	**Diterpenes test**	+	+	+
9	**Phytosterol**	-	-	+
10	**Phenol**	+	+	+
11	**Phlobatannins**	-	-	-
12	**Leucoanthocyanin**	-	-	-
13	**Anthoquinone**	-	-	-
14	**Cardiac glycosides**			
	a) Kellar- kellani test	+	+	+
15	**Carbohydrate test**			
	a) Iodine test	-	-	-
	b) Fehling test	+	+	+
	c) Benedict's test	-	-	+

Table 3 phytochemical analysis

2. Quantitative phytochemical screening

1) Total phenolic content determination

Total phenolic content of the *Curcuma longa* extract was quantified based on the linear equation obtained from the gallic acid standard curve was plotted at 0.2, 0.4,0.6,0.8,1.0 mg/ml concentration. Thus, the TPC value was recorded at a wavelength of 725nm and carried out triplicates. According to the calculated data, the TPC value (mg GAE/g) of the aqueous extract (R^2=0.8725) 0.46, acetone extract (R^2=0.9884) 1.22and methanol extract (R^2=0.9886) 1.52 respectively. This means that methanol is the best solvent to extract antioxidant content from the *Curcuma longa* leaves. (Fig.4-5)

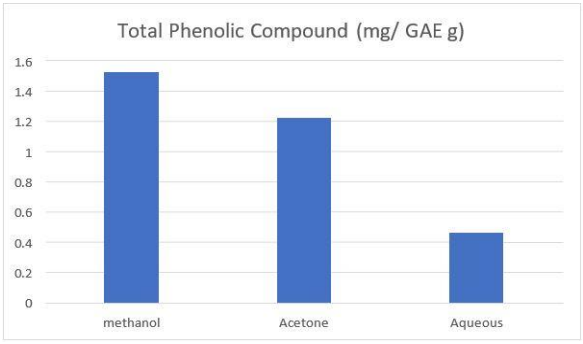


Fig 4: Total phenolic compound content (mg/GAE g)

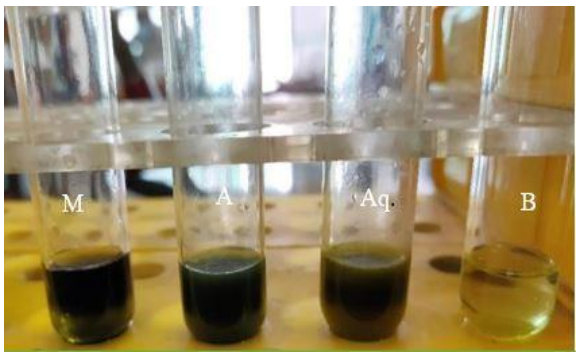


Fig 5: Total phenolic content

2) Total flavonoid content determination

Total flavonoid content of *Curcuma longa* leaf extract was calculated based on the linear equation obtained from quercetin standard and was plotted at 0.2,0.6,0.8,1.0 mg/ml concentration. The TFC value expressed as quercetin equivalent (mg QCE/gm). According to the experimental data the TFC value of the aqueous extract (R^2=0.9734) O.78, acetone extract (R^2= 0.9772) 1.34, and methanolic extract (R^2= 0.9838) 1.46 respectively. These result shows that methanol is the most suitable solvent to extract flavonoid contents in the *Curcuma longa* leaves. (Fig6-7)

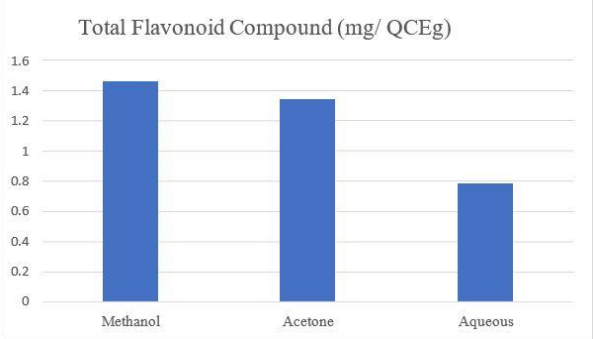


Fig.6: Total flavonoid compound content (mg /QCE g)

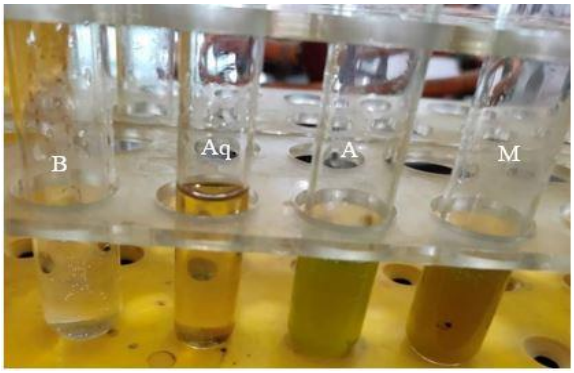


Fig.7: Total flavonoid content

Any medicinal plant requires detailed study prior to its use because; the therapeutic efficacy is absolutely dependent on the quality of the plant material used. The original and basic approach towards pharmacognosy includes study of morphological system, study of the cell structures. The organopathic evaluation provides the simplest and quickest means to establish the identify of a particular sample. The physiochemical parameters such as, ash value, extractive value of selected drug. The ash values are helpful in determining the quality and purity of a crude drug in powdered form. The extractive values are useful for the estimation of specific constituents, soluble in that particular solvent for the extraction [15].

Phytochemical screening revealed that flavonoid, cardiac glycosides, diterpenes, phenols compounds were obtained from the *Curcuma longa* leaves extract using methanol, acetone, and aqueous as a solvent. Cumarin were obtained from the *Curcuma longa* leaf extract using water and acetone as a solvent. Emodins, phytosterol, plobatannin, carbohydrate were obtained from the *Curcuma longa* leaves extract using water as a solvent. Water and methanol are polar solvents. These three solvents can attract flavonoids glycosides contained in the material.[15]

Chromatographic study

a. Thin layer chromatography

TLC was performed to separate and identified single or mixture of constituents in methanolic and acetonic extract. It was carried out for the identification of quercetin in the extract qualitatively. The bands were observed and RF value was calculated. The RF value of standard quercetin is 0.31 and the RF value of methanol and acetone extract is 0.34 and 0.32.

chromatographic study reveals that, quercetin present in *Curcuma longa* leaf extract. These results compared with research study of Bharathi S. It shows the Rf value of intense spot obtained 0.34 for methanol extract and 0.32 for acetone similar with Rf value of the standard quercetin 0.31.

b. Column chromatography

Column chromatography was performed to purify the mixture of constituents in methanolic and acetonic extract. The fraction was collected and OD was taken at 350nm (table 4-5) and (fig. 9-10) the highest OD obtained fraction was run for the TLC plate and observed under UV light.

Fraction No.	OD at 350nm	Fraction No.	OD at 350nm	Fraction No.	OD at 350nm
1	0.28	**27**	0.23	**43**	0.32
2	0.18	**28**	0.21	**44**	0.21
3	0.24	**29**	0.26	**45**	0.33
4	0.25	**30**	0.38	**46**	0.18
5	0.17	**31**	0.30	**47**	0.13
16	0.30	**32**	0.58	**48**	0.15
17	0.16	**33**	0.14	**49**	0.19
18	0.26	**34**	0.44	**50**	0.22
19	0.31	**35**	0.23	**51**	0.15
20	0.25	**36**	0.32	**52**	0.19
21	0.07	**37**	0.25	**53**	0.25
22	0.24	**38**	0.23	**54**	0.18
23	0.73	**39**	0.15	**55**	0.18
24	0.02	**40**	0.23	**56**	0.16
25	0.29	**41**	0.24		
26	0.27	**42**	0.22		

Table 4. collection of sample fraction from acetone extract

Fraction No.	OD at 350nm	Fraction No.	OD at 350nm
1	0.18	**8**	2.43
2	0.11	**9**	0.45
3	0.32	**10**	1.23
4	0.43	**11**	0.32
5	0.41	**12**	0.25
6	0.51	**13**	0.11
7	0.61	**14**	0.18

Table 5. collection of sample fraction from methanol extract

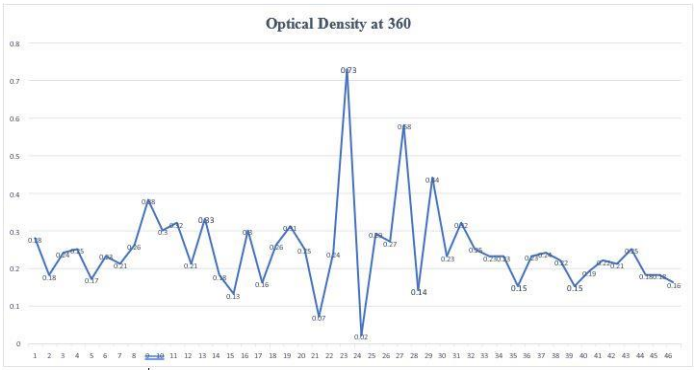


Fig 8 Column chromatography of Acetone fractions

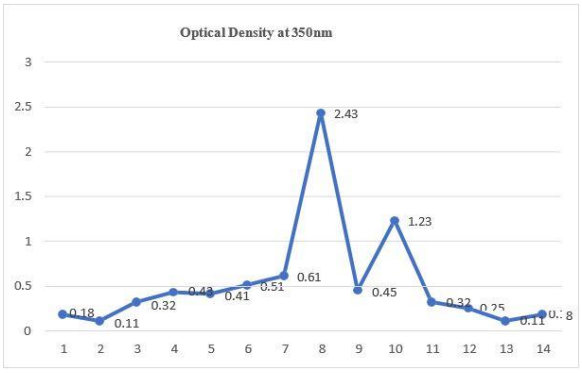


Fig 9 Column chromatography Methanol fractions

In this study the optimal extraction condition and the antioxidant properties of *Curcuma longa* leaf to develop it as a food source. In the particular, the *Curcuma longa* leaf methanol had high total phenolic compound (1.52mg GAE/g) and flavonoid (1.46 mg QCE/g) content. Comparing these results with other studies [13]. extracting turmeric leaves with various solvents, there is sight difference between extraction solvents related to antioxidant content. Ethanol extraction showed high total phenolic compound (283.56ug/mg) compared to methanol extraction (282.92ug/mg) and total flavonoid were also high with 70% ethanol (106.87ug/mg).

Antimicrobial assay

Detection of antibacterial activity of Methanol, acetone, and aqueous extract

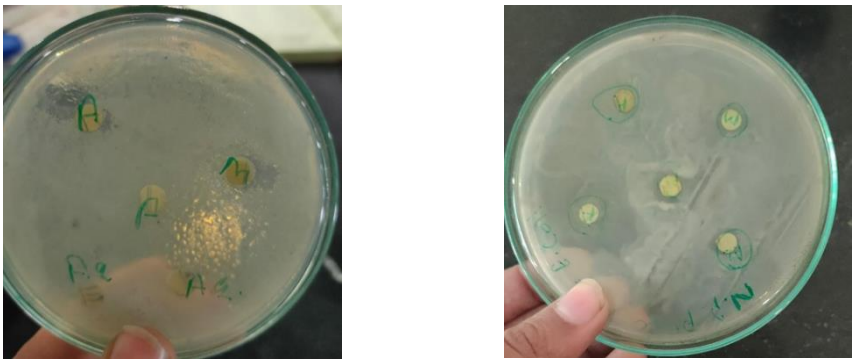
The *Curcuma longa* leaf extract has been tested for their antibacterial activities and an interesting antibacterial profile has been observed against gram positive (*B. thurengenesis*) and gram negative (*E.coli*). The *Curcuma longa* leaf extract showed enormous activity against the two bacteria tested. The activities of extract mentioned in the term of zone of inhibition(mm). (table 6) the zone of inhibition of methanol and acetone for *E.coli* and *B. thurengenesis* showed that it has antibacterial activity. (fig 10) aqueous extract does not showed zone of inhibition so, aqueous is lack of antibacterial activity.

***Curcuma longa* leaf extract**	**Zone of inhibition in mm**	
	E.coli	*B.thurengenesis*
Methanol	12mm	15mm
Acetone	10mm	11mm
Aqueous	-	-

Table 6 antibacterial activity of methanol, acetone and aqueous *Curcuma longa* leaf extract

Antifungal activity of methanol, acetone, and aqueous extract

Methanol, aqueous and acetone Curcuma longa leaf extract does not possess antifungal activity, as there is no zone of inhibition from when tested on *A. niger*.(fig 10 c)



a) **b)**

Fig 10 a) showing antibacterial activity of *B. thurengenesis* b) showing antibacterial activity of *E.coli*

In the present study showed that *Curcuma longa* leaf extract have antibacterial activity against *E.coli* and *B. thurengenesis* and the showed zone of inhibition on nutrient agar plate. Comparing these to study, *E.coli* and *B. thurengenesis* showes zone of inhibition on nutrient agar plate.

The study should be further explored for the selection of best extraction for folkloric use and Ayurvedic preparation. The extract should be investigated for Cytotoxicological and Genotoxicological parameters for safety standards.

CONCLUSION

Plants are main source of many components for the formulation of several drugs by the pharmaceutical industry. in these present investigations, various pharmacognostical parameters such as macroscopy and microscopy, preliminary phytochemical screening, TLC and Column chromatography, and Antimicrobial activity profiling were out which could be helpful in authentification of Curcuma longa. the results of the present study will also serve as reference material for the preparation of herbal monograph. further studies of the present investigation may recommend for the isolation of bioactive constituents and biological assay methods for the standard preparation.

REFERENCES

1. Petrovska B.B. Historical review of medicinal plant usage. Pharmacognocy review. 2012; 6(11): 1-5.
2. Vinoth S., Kanna P. R., Gurusaravanan P., Jayabalan N. Evaluation of phytochemical, antimicrobial and GC-MS analysis of extracts of Indigofra trivita subulate. International journal of Agricultural Research. 2011; 6(4): 358-367.
3. Martinez I., Perago Mj., Ros G. Nutritional importance of phenolic compounds in the diet. Archivos Latinoamericanos de nutrition. 2000; 5(2): 5-18.
4. Kelly EH., Anthony RT., Flavonoid antioxidant chemistry, metabolism and structure activity relationships. Journal of Nutritional Biochemistry.2002; 1(3): 572-584.
5. Kirtikar KR, Basu BD. Indian medicinal plants. Edn 2, International book distributors, Book seller and publisher, Dehradun, 1933; 2:9999.
6. Quality standards of Indian medicinal plants. Medical Plants Unit, ICMR, New Delhi, 2013,11.
7. Chauhan MG, Pillai APG. Microscopic profile of powdered drugs used in Indian systems of medicine Leaf drugs. Institute of P. G. teaching & research in Ayurveda, Gujrat Ayurved University, Jamner. 2007; 2: 106-107.
8. WHO Quality control methods for medicinal plants. Geneva, 2002; 28-31.
9. Government of India-Ministry of Health and Family Welfare. Indian Pharmacopeia, Edn. 1, India controller of Publications, New Delhi, 1996 II.
10. Harborne JB. Phytochemical Methods: A guide to Modern techniques of plant analysis, Edn 2, Chapman and Hall, London, 1984, 123-175.
11. Sawant R.S., Godghate A.G. Quantitative Phytochemical Screening Of Rhizome of *Curcuma longa* Linn. International Journal of Science, Environment and Technology, 2013; 2:634-641.
12. Sera Kim. Determination of *Curcuma longa* L. (Turmeric) Leaf extraction conditions using response surface methodology to optimize extraction yield and antioxidant content. Hindawi Journal of Food Quality. 2019; 1-8.
13. Heo S.J., Park E.J., Lee K.W, and Jeon Y.J. Antioxidant activities of enzymatic extracts from brown seaweeds. Bioresources Technology 2005; 96: 1613-1623.
14. Baba S.J. and Malik S.A. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of Arisaema jacquemontii Blume, Journal of Taibah University Medical Sciences, 2015; 4: 449-454.
15. Neyanila SK, Prakash YG and Gopal V. Preliminary Phytochemical and Pharmacognostical Standardization of Aerial Parts of Merremia Tridentata (Linn) Hallier. F. Convol Vulaceae. International journal of Pharmaceutical Research and Analysis, 2013; 3: 99-105.
16. Kokate CK, Purohit AR, Gokhale SB. Pathway to Screen Phytochemical nature of Natural Drugs. Pharmacognosy, 42nd ed, Nirali Prakashan Pune, 2008; 56-61.
17. Dordevic S, Cakic M, Amr S. The extraction of Apigenin and Luteolin from the Sage Salvia officianalis L from Jordan. The Scientific journal Facta Universities, 2000; 1: 87-93
18. Bharathi S, Devasena T, Arivarasan A, and Pachaiappan R. Extraction And Isolation Of Flavenoid Quercetin From The Leaves Of Trigonella Foenum Graecum And Their Antioxidant Activity, International Journal of Pharmacy and Pharmaceutical Science.2016;8:120-124.
19. Ilham LA., Herla R., Dwi S., and Dewi RS. Antimicrobial activity of turmeric leaf extract against Esherichia coli, Staphylococcus aureus, Shigella dysenteriae, and Lactobacillus acidophilus, International Conference on Agribussines, Food and Agro- Technology. 2008; 205: 1-8.