Published online in http://ijam.co.in ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 15 (1), 2024; 111-116

Incorporation of standardised extract of *Curcuma longa Linn* into phytosomes and its evaluation for *in vitro* Anti-inflammatory potential and Brine shrimp lethality assay

Research Article

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Abstract

Aim: To incorporate standardized *Curcuma longa* Linn extract into phytosomes and evaluate for *in vitro* anti-inflammatory and BSL bioassay. Method: The quality of the plant material was determined by various pharmacognostic parameters. The plant material was then subjected to maceration for extraction using ethanol: water as solvent followed by Soxhlet extraction. The resulting extract was subjected to phytochemical analysis to determine the presence of plant metabolites. The drug and excipients compatibility was evaluated by FTIR study. Furthermore, using the thin film hydration approach, a new lipid-based phytosome was prepared. *In vitro* anti-inflammatory and brine shrimp lethality tests were performed on prepared phytosome. Results: Moisture content, total ash, acid insoluble ash, water soluble ash values, aqueous, alcohol, and petroleum ether extractive values are all found to be within limits. The phytochemical analysis validated the existence of alkaloids, tannins, resins, carbohydrates, proteins, flavonoids, and saponins. The compatibility study demonstrates the compatibility of excipients with drugs. Thin film hydration technique was employed successfully to prepare the phytosomes containing *Curcuma longa* linn extract. *In vitro* anti-inflammatory activity revealed that prepared phytosome could serve as natural based therapeutic option for anti-inflammatory potential. Brine shrimp lethality assay also confirmed the bioactivity of prepared phytosomes. Conclusion: The method used for standardization can be used to aid with plant identification and quality analysis of *Curcuma longa* Linn for future research. It can be inferred from the findings that phytosomes loaded with *Curcuma longa* Linn extract exhibited promising anti-inflammatory and cytotoxic effects.

Keywords: Phytosomes, Nanoformulation, *Curcuma longa Linn,* Anti-inflammatory, Brine Shrimp Lethality assay.

Introduction

Primitive civilisations viewed natural based products as valuable and utilised them to treat human diseases(1).Plants have long been used by humans as a source of food and everyday essentials, including for the production of paper, building materials, finely ground spices, and uses for the prevention and treatment of various ailments(2). Due to its moderate therapeutic impact and minimal side effects, herbal medicine has gained significant interest and has generated a wealth of practical knowledge over the course of several millennia(3). These herbal based treatments are becoming increasingly popular due to a variety of factors, including reduced adverse reactions, enhanced patient tolerability, reduced costs, and long-standing history of use(4). The utilization of medicinal plants with therapeutic benefits continues to provide the basis for a variety of traditional medicinal systems. Plant-derived products play an integral part in human growth; in addition to the three fundamental necessities of human existence, health is an essential need that is heavily dependent on natural resources (5). Since the second millennium BC, the dried rhizome of the perennial herb *Curcuma longa Linn* member of the zingiberaceae family has been used as medicine (6). It has a wide range of pharmacological effects, including antioxidant, anti-inflammatory, anti-cancer, anti-diabetic, anti-arthritic, and anti-microbial properties (7).The yellow color is brought on by curcumin. It also contains phellandrene, sabinene, cineol, borneol, zingiberene and sesquiterpenes (8). Curcumin's therapeutic applicability has been hindered by its poor water solubility, short half-life, and low bioavailability resulting from oral administration despite its potential therapeutic efficacy and favourable safety profile. Curcumin has an extremely fast first-pass metabolism and is quickly excreted from the body. This results in a relatively short retention period in circulation. Over the past few decades, different formulations have been

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developed to overcome these disadvantages. In these studies, the formation of solid dispersions complex was achieved using a variety of methods, including the use of cyclodextrin-based compounds, copolymeric micelles and polymeric nanoparticles as well as liposomes, phospholipid complexes, and self-emulsion. (9). Phytosomes are nano drug delivery systems that follows a liposome design in which encapsulated phytochemicals and the phospholipids that make up the liposome interact at the molecular level. Chemical bonds between the entrapped drug and the phospholipid provide benefits like high entrapment efficiency, a higher stability profile, and increased gastrointestinal absorption, all of which improve bioavailability and boosts the pharmacological effects (10).

Non-steroidal anti-inflammatory drugs (NSAID) have been demonstrated to be effective in the treatment of pain and inflammation. However, utilisation of these drugs has been linked to a range of undesirable effects. *Curcuma longa Linn* is a well-known medicinal plant with anti-inflammatory activity. (11). The brine shrimp lethality bioassay is commonly employed in the evaluation of the toxicity of heavy metal, pesticides, medicinal products, particularly natural plant extracts. This bioassay serves as a preliminary toxicity test for further experimentation on mammalian animal models. (12).

The current study attempted to standardise *Curcuma longa* Linn by employing pharmacognostical evaluation and formulation into phytosomes for *in vitro* anti-inflammatory and brine shrimp lethality assay.

Materials and Methods

Raw material and chemicals

The rhizomes of *Curcuma longa* were obtained from local Ayurveda pharmacy. All other chemicals and analytical grade reagents were sourced from KLE College of Pharmacy, Belagavi.

Processing of raw material

The dried rhizomes of *Curcuma longa* was transformed into a powdered state and kept in a tightly sealed receptacle until it was needed again.

Pharmacognostical study

Organoleptic evaluations were conducted on the collected raw material. Additionally, physicochemical evaluations were conducted to assess moisture content, ash values and extractive values (13).

Extraction

Cold maceration was the primary method of extracting thermo-labile constituents from dried powdered material with hydro alcoholic solvent in the ratio of 70:30 for 24 hrs. Marc from maceration was additionally subjected to Soxhlet extraction for a period of 24 hours. Both the maceration and Soxhlet extraction filtrates were mixed and concentrated by evaporation under reduced pressure using rotary evaporator (14).

Phytochemical investigation

The final extract was then subjected to a phytochemical test for qualitative identification of Alkaloids, Glycosides, Tannins, Resins, Carbohydrates, Flavonoids, Proteins and Saponins (13).

Compatibility study

FTIR was used to investigate the compatibility of extract with other excipients. For compatibility studies, the FTIR spectrum of extract, soya lecithin, cholesterol, and physical combination is used. (15).

Phytosome formulation

Thin film hydration was employed for the preparation of phytosomes. Briefly, 2:1 ratio of soya lecithin (100mg) and cholesterol (50mg) were dissolved in chloroform (10ml) and 50mg of the extract were dissolved in methanol (10ml) in two separate beakers. The solvents from both beakers were poured into a round bottom flask, and evaporated in the rotary evaporator (40^{o}C at 180 rpm) until all solvent evaporates and a thin layer forms on the RBF. The flask was stored in the refrigerator for 24 hours. A 1:1 ratio of ethanol and water was employed to evaporate the film for 1 hour at 40^{o}C in evaporator. Subsequently, the particle size was reduced by sonication for 30 minutes following the production of the phytosomal suspension. (15, 16, 17).

Brine Shrimp Lethality assay

A container filled with saline solution was used to hatch the eggs of brine shrimp under suitable conditions of temperature, light, and aeration. The eggs hatched into nauplii (larval stage of brine shrimp) within a day. A series of test solutions with different concentrations of the sample were prepared by diluting them in the solvent. 10 brine shrimp nauplii were added to each container, including the control using a micropipette. The nauplii are allowed to swim freely in the test solutions. The containers were incubated under suitable conditions for 24 hours. The temperature and lighting conditions were maintained. After the incubation period, the containers were examined to determine the number of surviving brine shrimp nauplii in each concentration and control. The lethality or mortality rate is calculated by counting the number of dead nauplii (5, 18, 19).

In vitro anti – inflammatory activity

The anti-inflammatory activity of the test sample was evaluated using the egg albumin method. This approach is frequently employed to assess the inhibitory effect of substances on protein denaturation, which is associated with inflammation (20).The experiment involved preparing a series of test solutions with varying concentrations of the test sample. A control solution without the test sample was also prepared. Egg albumin solution was used as a model protein for inducing denaturation, simulating the inflammatory response. To the test solution, egg albumin (0.2ml), test sample (2ml), phosphate buffer (2.8ml) of pH 6.4 was added in each test tube. The mixtures were mixed and

incubated for 15 min at 37°C under controlled conditions to allow denaturation of the egg albumin. After the incubation period, the mixtures were heated at 70°C temperature for 5 min to induce further denaturation. The mixtures were cooled and absorbance were noted at 660nm using UV-Visible spectrophotometry. The percentage inhibition was calculated by using below formula (21, 22).

$$Percentage\ Inhibition = \frac{Absorbance\ of\ Control - Absorbance\ of\ Test}{Absorbance\ of\ Control} \times 100$$

Results

Pharmacognostical study

Organoleptic study

The organoleptic assessment is a fundamental element in the process of accurately identifying plant material. Below are some of the organoleptic features of the *Curcuma longa*.

Figure No.1: *Curcuma longa*

Table No.1: Organoleptic evaluation of *curcuma longa*

Characters	Observation
Colour	Yellow or yellowish brown
Odour	Aromatic and characteristic
Taste	Slightly bitter
Shape	Irregular, finger shaped
Surface	Smooth, slightly rough
Texture	Hard
Fractures	Short

Physicochemical investigation of *Curcuma longa*

The physicochemical properties are the building blocks of any plant-based material standardization. This text outlines the physicochemical properties of the *Curcuma longa*, including moisture content, ash value, as well as extractive values.

Table No.2: Physicochemical investigation of *Curcuma longa*

Sr. No	Physicochemical parameters (%w/w)	Test	Result (%w/w)
1	Moisture Content	Gravimetric method	9
2	Ash value	Total ash	10
		Acid insoluble ash	9.05
		Water soluble ash	7.39
3	Extractive values	Water soluble	6
		Alcohol soluble	12
		Petroleum ether soluble	3

Extraction

Extraction was carried by maceration followed by Soxhlet extraction. The yield of resultant hydro alcoholic extract was found to be 12.70%.

Figure No. 2: *Curcuma longa* extract

Phytochemical analysis

The qualitative phytochemical analysis of the *Curcuma longa* rhizomes revealed the presence of the following metabolites.

Table No 3: Phytochemical analysis

Phytochemicals	Results
Alkaloids	+
Glycosides	-
Tannins	+
Resins	+
Carbohydrates	+
Proteins	+
Flavonoids	+
Saponins	+

('+'represents positive results and '-' represents negative results)

Compatibility study

The following table outlines the FTIR spectrum for extract, soya lecithin, cholesterol and physical mixture.

Table No. 04: Compatibility study by FTIR

Group	Soya lecithin	Cholesterol	Extract	Physical Mixture
C=C stretch	1615.45	1465.96	1559.51	1581
C=O stretch	1734.08		1603.35	1715.76
C-H stretch	-	2848.98	2809.44	2850.91
-OH stretch	-	2932.89	3229.94	2930.00 -2924.21

Figure No. 3: FTIR spectrum of *Curcuma longa*

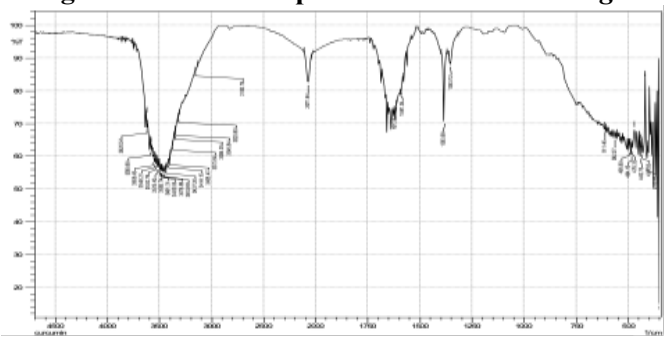


Figure No. 4: FTIR spectrum of Cholesterol

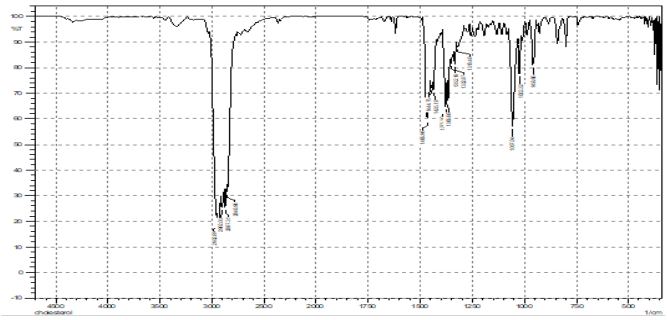


Figure No. 5: FTIR spectrum of soya lecithin

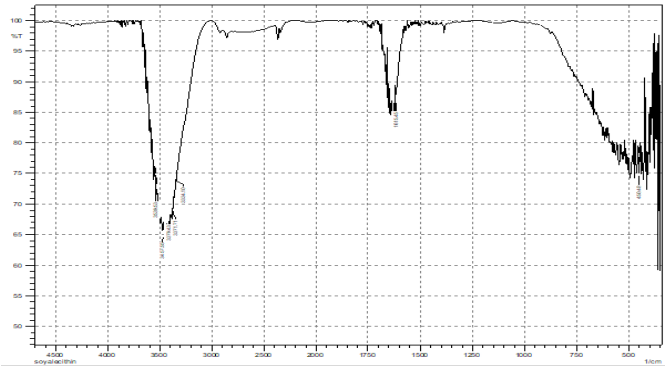
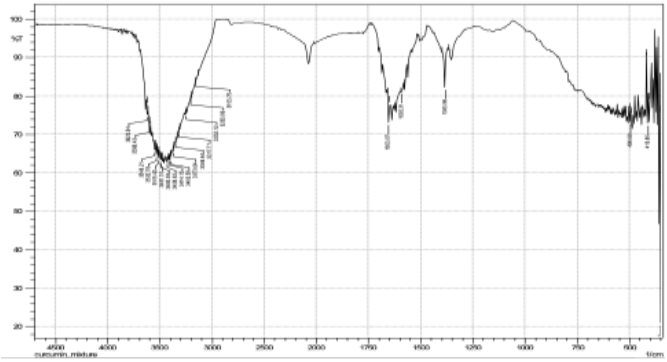


Figure No. 6 : FTIR spectrum of physical mixture

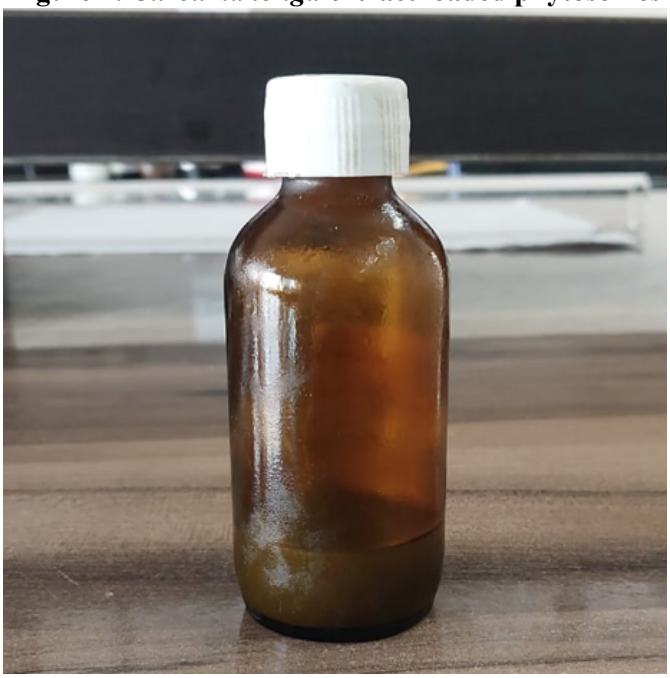


The similarity of the obtained peaks in the FTIR spectra of the extract and mixture suggests a high degree of compatibility between the drug and excipients. The presence of similar peaks indicates that the molecular components and functional groups present in the extract are retained in the mixture, suggesting that they are compatible with each other. This compatibility is important as it implies that the components of the extract can coexist and interact effectively in the mixture, potentially leading to desired outcomes. The matching peaks in the spectra provide evidence of a harmonious combination, supporting the notion that the extract and excipients are compatible.

Phytosome formulation

Thin film hydration technique was used to prepare *Curcuma longa* extract loaded phytosomes. The prepared phytosomes were well prepared and subjected for in vitro anti-inflammatory and Brine shrimp lethality assay. Figure 7 represents *Curcuma longa* extract loaded phytosomes.

Figure 7: *Curcuma longa* extract loaded phytosomes



Brine Shrimp Lethality assay

The cytotoxicity of prepared phytosome was evaluated by BSL assay. The results obtained for the BSL assay are depicted in the table below:

Table no.5: Results of BSL Assay

Concentration (µg/ml)	No. of Brine Shrimps alive (Out of 10)	No. of dead nauplii	% Death
Control	10	0	0
10	5	5	50
100	0	10	100
1000	0	10	100

Anti – inflammatory activity

The results obtained for anti – inflammatory activity carried out by using egg albumin method is as follows:

Table No. 6: Anti - inflammatory activity	
Concentration (µg/ml)	**Percentage inhibition (%)**
Control	0
Diclofenac	71.22
10	14.24
100	34.89
1000	60.22

Discussion

Curcuma longa is a massive plant with numerous medicinal applications due to the presence of a diverse range of active phytoconstituents. An effort has been made in this work to standardise *Curcuma longa* and formulate novel phytosomes containing *Curcuma longa* extract for its anti-inflammatory activities. The quality of the plant material was evaluated by pharmacognostic standardisation parameters. The organoleptic assessment is used as a benchmark to accurately identify plant material and to eliminate any undesired contaminants. In order to ensure the highest level of pharmaceutical stability, it is generally recommended to maintain a low moisture content (23).The moisture content for *Curcuma longa* was found be 9% w/w which indicates good stability of product. Ash value is the primary criterion for assessing the quality of herbal medicinal products. Ash levels typically indicate the presence of inorganic compounds present in herbal medicinal products, including phosphate, carbonate, and silicate. These are important factors in determining the purity and quality of herbal medicines (24). An extractive value, which varies depending on the solvent, verifies the existence of active components. When the water-soluble, alcohol-soluble, and petroleum ether-soluble extractive values of the drugs were compared, it was discovered that the percent water-soluble and alcohol-soluble extractive values were higher than the petroleum ether; this indicates that the plants contained more water and alcohol soluble contents (5). The results demonstrate that all physicochemical variables are within acceptable parameters. Plants contain alkaloids, tannins, resins, carbohydrates, proteins, saponins, and flavonoids, according to phytochemical analysis. Compatibility study confirms that drug and excipients used for phytosome preparation are compatible with each other. Further, using the thin film hydration approach, a new lipid-based phytosome loaded with *Curcuma longa* extract was developed. The resultant phytosomes loaded with *Curcuma longa* extract was subjected in vitro biological activity evaluation using standard methods. Based on the results, prepared phytsomes showed promising anti-inflammatory activity which was compared with standard dichlofenac. Based on BSL bioassay results, prepared phytosomes showed better cytotoxicity which will give idea for further experimentation on mammalian animal models. Some formulation issues have been reported with conventional dosage forms and low bio-availability of phytochemicals which are greatest obstacles in therapeutics of phytopharmaceuticals. These obstacles can be overlooked by lipid based new drug delivery system which may give promising outputs in the sense of bioavailability.

Conclusion

Curcuma longa has gained significant attention for its potential health benefits. It contains a bioactive compound called curcumin which has anti-cancer, anti-inflammatory, and antioxidant effects. Curcumin is responsible for the distinctive yellow colour of turmeric and is the key component that contributes to its medicinal properties (25). Research studies have demonstrated the potential of *Curcuma longa* in various therapeutic applications, particularly in the field of inflammation. Curcumin exerts its anti-inflammatory effects by modulating multiple molecular pathways involved in the inflammatory response. It has been shown to inhibit the activity of various pro-inflammatory enzymes and cytokines, thereby reducing inflammation and alleviating associated symptoms (26). Although curcumin has therapeutic potential and a positive safety profile, its clinical use is limited due to its low water solubility and short half-life, as well as low bioavailability after oral administration. Traditional dosage forms, on the other hand, face various issues such as low absorption, toxic effects, solubility, and so on. In order to overcome these challenges, novel lipid-based nano formulations have gained popularity in the effective therapeutic treatment module by enhancing bioavailability (27). The current study attempted to develop and analyse phytosomes loaded with *Curcuma longa* extract and its evaluation on anti-inflammatory activities and cytotoxicity assessment by BSL Bioassay. The current work also includes the development of quality control parameters for *Curcuma longa*. As a result, this method can be used for future plant identification and research. Prepared phytosomes demonstrated promising biological properties in terms of anti-inflammatory activity and cytotoxicity by BSL bioassay. This work can be considered as stepping stone in new drug delivery systems based on herbal products which in terms provide numerous advantages over conventional dosage forms. In order to draw further inferences, further *in vivo* studies should be planned.

Acknowledgement: The authors are heartily thankful to the Principal, KLE College of Pharmacy, Belagavi for providing necessary facilities.

Abbreviations:

BSL: Brine Shrimp Lethality Assay

FTIR: Fourier transform infrared Spectroscopy

RBF: Round Bottom Flask

Ml: Milliliter

UV-Visible Spectroscopy: Ultraviolet Visible Spectroscopy

NSAID Non-steroidal anti-inflammatory drugs

µg/ml: Microgram per Milliliter

w/w: Weight by Weight

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