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Quality standardization, Phytosome formulation and *in vitro* antioxidant activity of *Moringa oleifera* Lam: An Ayurvedic medicinal plant

Research Article

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Abstract

Aim: To formulate and evaluate *Moringa oleifera* Lam loaded phytosome for *in vitro* antioxidant activity. Method: The plant material was analyzed for its quality. The plant material was subjected for extraction by using maceration followed by Soxhlet extraction using ethanol: water as solvent. The resultant extract was subjected for phytochemical investigations. Furthermore, novel phytosome were prepared by thin film hydration method. The prepared phytosomes were analysed for particle size and entrapment efficiency. The optimized formulation (F3) was subjected for *in vitro* antioxidant activity. Thin Layer Chromatography was performed using Quercetin as standard and Methanol: Water as mobile phase. Results: The organoleptic evaluation revealed the plant is green, aromatic odour, slightly bitter taste erect stems, thick grey bark, flowers are white. Physicochemical parameters such as Moisture content, Total, Acid Insoluble, Water soluble ash, Aqueous, Alcohol and Petroleum ether extractive values are found to be 8.5, 10.5, 5, 12.5, 12.5 and 2.5% respectively. The phytochemical investigation confirmed the presence of Alkaloids, Glycosides, Steroids, Terpenoids and Phenols. The compatibility study confirms the excipients and drugs are compatible with each other. Among four prepared phytosomes with different ratios, formulation 3 exhibited smaller particle size such as 141nm and entrapment efficiency as 81%. Hence, formulation 3 is subjected for further antioxidant activity. With the IC_{50} value of 17.82µg/ml, formulation 3 showed promising antioxidant activity which was compared with standard ascorbic acid with IC_{50} 33.53 µg/ml. Conclusion: Based on the results, it can be concluded that novel *Moringa oleifera* Lam phytosomes exhibited promising Antioxidant properties.

Key Words: Phytosomes*, Moringa oleifera Lam,* Antioxidant, Standardization, Bioavailability.

Introduction

Early civilizations regarded medicinal plants as precious and used them to remedy human ailments. (1). These are gaining popularity due to several advantages, including fewer side effects, improved patient tolerance, lower cost, and acceptance due to a long history of use(2, 3). Medicinal plants with therapeutic properties continue to serve as the foundation for various traditional medicines. Plant-based products play an indistinguishable role in human development; aside from the three basic needs of human life, health is another important need that is highly reliant on natural resources(4). For various reasons, the popularity of herbal medicine will continue to grow around the world, necessitating the urgent need for appropriate and sufficient information on herbal medicine, particularly that focusing on important topics such as benefits, efficacy, safety, toxicity, research and development, formulation, regulation, analytical techniques, quality control, and economic significance(5).

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Moringa oleifera Lam is a cruciferous plant that belongs to the genus *Moringa* and family *Moringaceae.* It is also known as the 'horseradish tree' or 'drumstick tree,' native to Sub-Himalayan region of northern India (6, 7). The plant have reported the presence of moringine, moringinine, vanillin,bayrenol, indole acetic acid, indole acetonitrile, benzylisothiocynate, pterygospermine, cartotene, flavonoids, polysaccharides, protein components, fatty acids, spirochein, and pterygospermine (8). *Moringa oleifera* Lam exerts diverse therapeutic properties such as antihyperthyroidism, natural coagulant, antitumor, anti-inflammatory, antiulcer, antispasmodic, antihypertensive, antidiabetic, hepatoprotective, antibacterial, and antifungal properties due to presence of wide range of phytoconstituents (9).

The extremely low absorption rate and poor penetration across biological barriers are the biggest obstacles in therapeutic potential of phytochemicals (10). The traditional dosage forms are having several limitations. A unique drug delivery method that overcomes the drawbacks of conventional drug delivery methods is termed as a novel drug delivery system (11). Phytosomes are unique dosage form that improves the absorption and therapeutic efficacy of herbal medicines by means of novel drug delivery system. Phytosomes creates a link between the traditional and the novel drug delivery system for incorporating standardized phytoconstituents into phospholipids to produce lipid compatible molecular complexes to increase absorption and bioavailability of poorly absorbed phytoconstituents and

to obtain higher therapeutic effects (12). Recent reports suggests that antioxidants produced from plants have the ability to scavenge free radicals and it is of great therapeutic value in treating diseases caused by free radicals, such as diabetes, cancer, neurodegenerative disease, cardiovascular disease, ageing, gastrointestinal diseases, arthritis etc. On the other hand, synthetic drugs have reported some harmful as well as mutagenic effects. Hence, plant based therapeutic agent with potent antioxidant activity will be the promising tool for treating diseases caused by free radicals(13).

In this work, an effort has been made to standardize and formulate novel phytosomes containing *Moringa oleifera* Lam extract and its evaluation for *in vitro* antioxidant properties. The current investigation will be connecting link between traditional and novel drug delivery system. Furthermore, the standardization parameters will guide for the correct identification and quality measures for the medicinally important *Moringa oleifera* Lam plant.

Materials and Methods

Plant material and Chemicals

Fresh stem and leaves of *Moringa oleifera* Lam was obtained from a local area in Belagavi, Karnataka. Dr. Harsha Hegade, ICMR, Belagavi, Karnataka, authenticated it, and voucher specimen number RMRC-1695 was deposited in the herbarium. DPPH (2, 2-diphenyl-1-picrylhydrazyl) and all other analytical grade chemicals and reagents were obtained from KLE College of Pharmacy, Belagavi.

Processing of plant material

The freshly collected plant material was thoroughly cleaned under running tap water and it was subjected for shade drying. Furthermore, the dried plant material was converted into powder form and stored in well closed container until further use.

Extraction of plant material

Dried powdered material was first subjected to cold maceration to extract thermolabile constituents with 70% v/v ethanol for 24 hr. Extract was further subjected for Soxhlet extraction. The resultant filtrate from both maceration and Soxhlet extraction were combined and concentrated using a rotary evaporator at 40°C under reduced pressure (14).

Pharmacognostical study

The organoleptic evaluation was performed on freshly collected plant material. On powdered plant material, physicochemical evaluations such as moisture content, ash value, and extractive value were performed (15).

Phytochemical Investigation

The resultant extract was subjected for phytochemical analysis for qualitative detection of Alkaloids, Flavonoids, Phenols, Terpenoids and Sterols (16).

Compatibility study

The compatibility study of extract along with other excipients were analyzed by FTIR. The FTIR spectrum of extract, soya lecithin, cholesterol and physical mixture is considered for compatibility study. Also DSC thermogram for extract was studied.

Phytosome formulation

Phytosomes were prepared by using different ratios of soya lecithin and cholesterol by thin film hydration method. The drug was dissolved in methanol, while the soya lecithin and cholesterol were dissolved in dichloromethane. The above mixture was placed in a round bottom flask and evaporated through a rotary evaporator at 40°c and 180 rpm until all of the solvent had evaporated and a thin layer had formed on the RBF. For up to 24 hours, the flask was kept in the fridge. Mixture of ethanol and water (1:1) was used to hydrate the film for 1 hour at 40°c in a rotary evaporator. The particle size was decreased by sonication for 30 minutes after the producing the phytosomal suspension. Table 1 summarizes the different ratios of formulation components (3).

Table No: 1 Preparation of Phytosomes

Formulations	Cholesterol (mg)	Soya lecithin (mg)	Drug (mg)
F1	15	10	150
F2	20	15	150
F3	25	20	150
F4	30	25	150

Thin Layer Chromatography

TLC was performed on all prepared formulation with Quercetin as a standard. Precoated TLC plates was used in TLC analysis. The freshly prepared sample and standard were applied on precoated TLC plates with capillary tubes and kept in developing chamber with solvent system as Methanol: Water (7:3). After development, the plates were gently drawn and allowed to air drying and observed under UV chamber (17).

The Rf value were calculated and compared with standard.

$$\text{Rf value formula:} \quad \frac{\textbf{Distance traveled by solute}}{\textbf{Distance travelled by solvent}}$$

In vitro antioxidant activity

The prepared phytosomes with smaller particle size were analyzed for *in vitro* antioxidant activity by DPPH radical scavenging assay. Briefly, working dilution were prepared and analyzed for its activity. In the test tube, 1ml of test solution and 3ml of standard DPPH reagent (4mg prepared in 100ml of methanol) were added .Similarly, methanol was added to the DPPH solution to provide the control. The test tubes containing the test solutions were correctly packed and maintained at room temperature in the dark for 30 mins. After that, the absorbance was measured at 517nm.The IC_{50} was computed using SPSS-20 software (18, 19).

$$Radical\ scavenging\ activity(\%) = \frac{Abs\ Control - Abs\ sample}{Abs\ control} \times 100$$

Results

Pharmacognostical study

Figure No.1: Moringa oleifera Lam

Organoleptic study

The organoleptic evaluation is the important tool for the exact identification of plant material. The organoleptic characters of *Moringa oleifera* Lam is depicted below:

Table No.2: Organoleptic evaluation

Observation	Characteristics
Color	Green
Odor	Aromatic
Taste	Slightly sweet and bitter
Leaves	Tripinnate between 30 and 60 mm in length,
Flowers	Flowers are yellowish white in color about 2
Fruits	Brown dehiscent capsules up to 45 cm in
Extra features	Stems erect, thick gray bark

Physicochemical investigation of *Moringa oleifera* Lam.

The physicochemical parameters are the important tools for the standardization of any plant based materials. The following is a representation of the physicochemical characteristics of *Moringa oleifera* Lam, including its moisture content, ash value, and extractive values.

Table No.3: Physicochemical investigation of *Moringa oleifera* Lam

Sr. No	Physico-chemical parameters (%w/w)	Test	Result (%w/w)
1	Ash value	Total ash	10
		Acid insoluble ash	5
		Water soluble ash	5
2	Extractive values	Water soluble	12.5
		Alcohol soluble	12.5
		Petroleum ether soluble	2.5
3	Moisture content	Gravimetric method	8.5

Phytochemical analysis

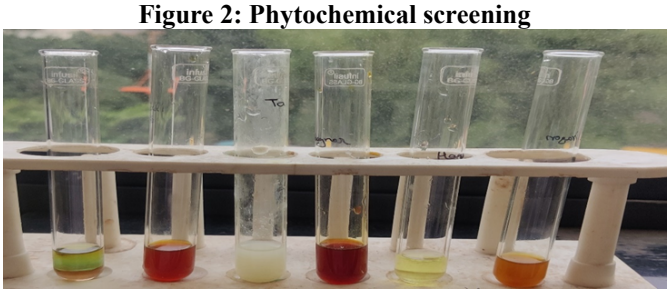
The qualitative phytochemical analysis of whole plant of *Moringa oleifera* Lam showed the presence of following metabolites.

Table No 4: Phytochemical analysis

Phytochemicals	Extract
Phenols	+
Terpenoids	+
Sterols	+
Alkaloids	+
Flavonoids	+

('+'indicates positive results and '-' indicates Negative results)

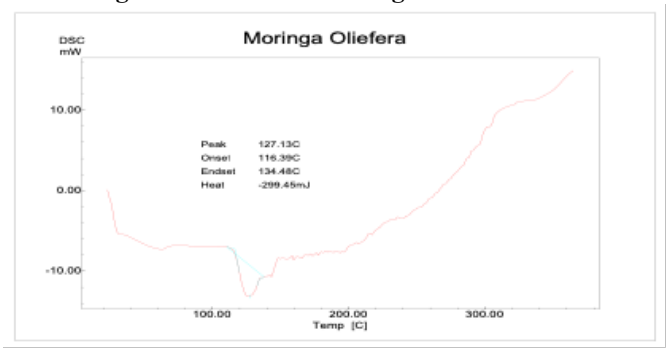
Figure 2: Phytochemical screening



Preparation of Phytosomes

The FTIR spectrum of extract, soya lecithin, cholesterol and physical mixture is along with DSC thermo gram for extract is given below:

Figure No. 3: DSC thermogram for extract



Compatibility study

The compatibility study of extract along with other excipients were analyzed by FTIR. It is depicted below:

Table No. 5: FTIR Stretching

Functional group	Soya lecithin	Cholesterol	Extract	Physical mixture
C=C Stretch	1615.45	1465.96	-	1465.00
OH Stretch	-	2932.89	2916.49	2930.00-2924.21
C-H Stretch	-	2848.98	2848.98	2849.95
C=O Stretch	1734.08	-	1748	1734.08

Figure No. 4 : FTIR spectra of extract

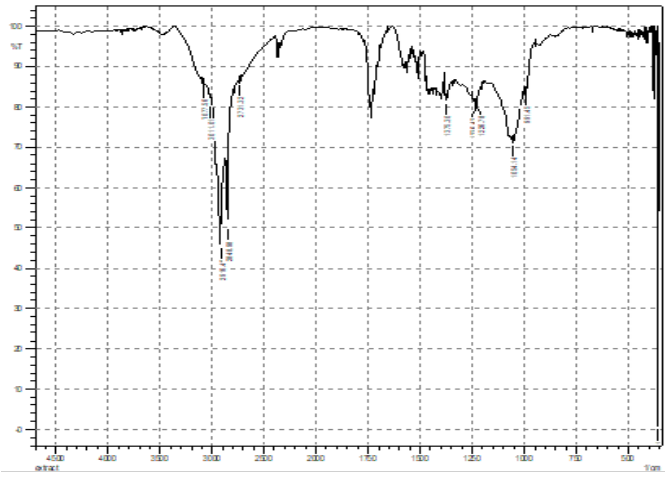


Figure No. 5: FTIR spectra of Cholesterol

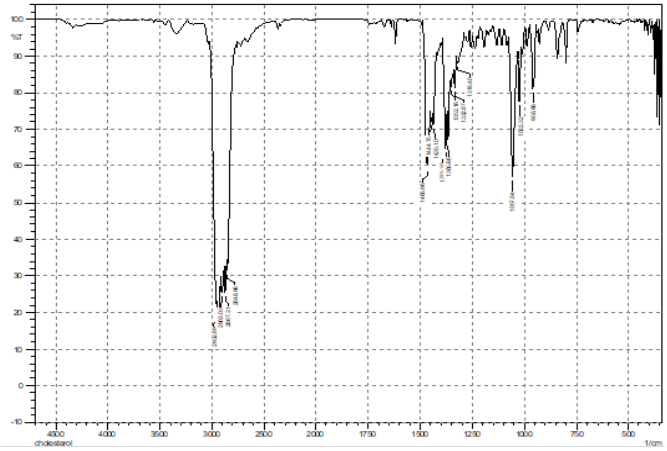


Figure No. 6: FTIR spectra of soya lecithin

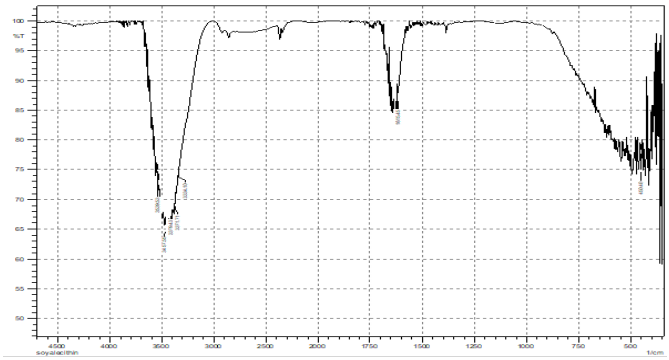
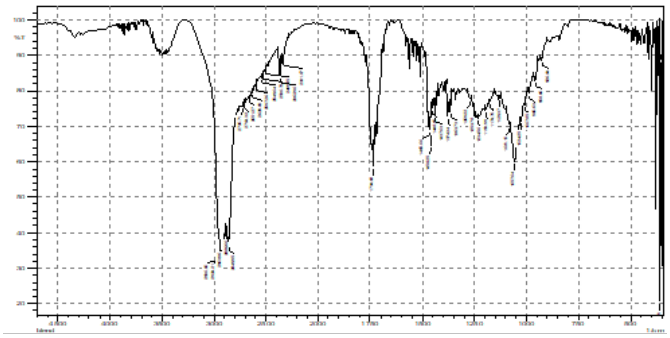


Figure No. 7: FTIR spectra of physical mixture



Phytosome formulation

The prepared phytosomes were analyzed for its particle size and polydispersability index. Formulation no 3 exhibited smaller particle size with 0.7 polydispersability index. Furthermore, formulation 3 was evaluated for entrapment efficiency which is found to be 81 %.The results are depicted below:

Figure No. 8: Phytosomes loaded with *Moringa oleifera* Lam

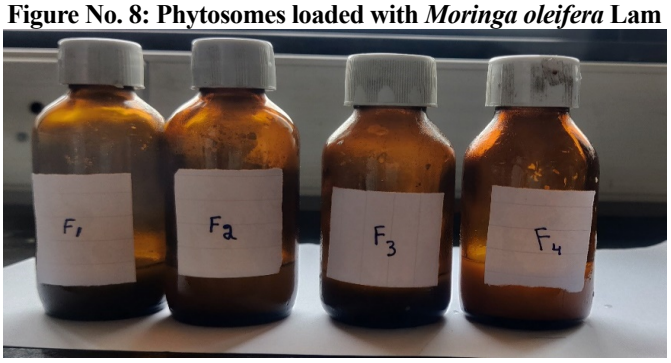


Table No 6: Particle size, PDI and Entrapment efficacy

Formulation	Particle size (nm)	PDI	Entrapment efficiency
F1	241	0.7	**F3**= 81%
F2	209	0.9	
F3	141	0.7	
F4	255	0.5	

TLC

TLC was performed for phytosomes containing *Moringa oleifera* Lam extract. Quercetin is used as standard. Methanol: water (7:3) was used as mobile phase. The Rf value for prepared phytosomes and standard quercetin is depicted below:

Table No 7: Thin Layer Chromatography

Sr No	Sample	Rf value
1	Quercetin (Standard)	0.77
2	F1	0.78
3	F2	0.76
4	F3	0.77
5	F4	0.79

Figure No. 6: Thin Layer Chromatography

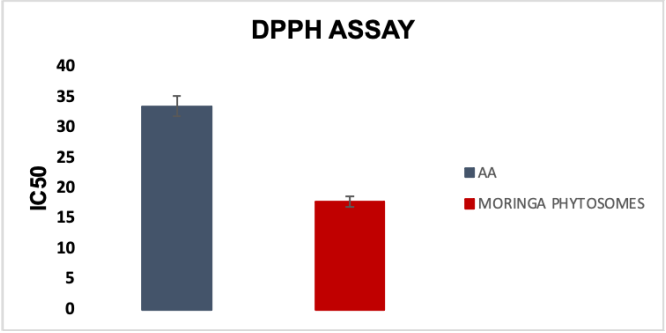
Antioxidant activity

The *in vitro* antioxidant activity was carried out as per previously reported procedure. The assay was carried on formulation 3 with smaller particle size and it was compared with standard ascorbic acid. The percent inhibition and IC_{50} value for ascorbic acid and phytosome loaded with *Moringa oleifera* Lam extract is depicted in Table No 8 and Figure No 7 Respectively.

Table No 8: Antioxidant activity

Sr. No	Concentration (µg/ml)	Percent Inhibition (%)	
		Standard	Phytosomes
1	2	58.47	57.56
2	4	71.02	59.67
3	6	77.23	73.73
4	8	86.25	74.38
5	10	98.11	87.05
IC_{50} µg/ml		33.53	17.82

Figure No 7: IC_{50} Values



Discussion

Moringa oleifera Lam is an enormous plant having several medicinal utilities due to presence of wide range of active phytoconstituents. In this work, an effort has been

made on standardization of *Moringa oleifera* Lam and formulation of novel phytosomes containing *Moringa oleifera* Lam extract for its *in vitro* antioxidant properties. The pharmacognostical standardization parameters were assessed in terms of quality. The organoleptic evaluation is benchmarker for the correct identification of plant material and to avoid any contaminants. One of the main causes of the degradation of pharmaceutical products is moisture. Low moisture content is always preferred for greater pharmaceutical stability (20). The ash value is the most essential metric for determining the quality of herbal medications. The ash levels often represent the inorganic residues found in herbal medicines, such as phosphates, carbonates, and silicates. These are significant indicators of the quality and purity of herbal drugs (21). Depending on the solvent, an extractive value confirms the presence of active components. When the water-soluble, alcohol-soluble, and petroleum ether-soluble extractive values of the drugs were compared, it was determined that the percent water-soluble and alcohol soluble extractive values were higher than the petroleum ether; this indicates the presence of more water and alcohol soluble contents in the plants (4, 20). According to the findings, all physicochemical parameters are confirmed to be within tolerance. Phytochemical analysis has revealed that plants include lipids, phenols, terpenoids, sterols, alkaloids, and flavonoids. Furthermore, novel lipid based phytosome loaded with *Moringa oleifera* Lam extract was prepared by thin film hydration technique. The compatibility study reveals the drug and excipients are compatible with each other. Four formulations of phytosomes were successfully prepared using different combination of soya lecithin and cholesterol. Among the four formulations, with particle size of 141 nm and polydispersability index of 0.7 and entrapment efficiency of 81% formulation 3 (F3) exhibited better results in terms of characterization of phytosomes. Thin layer chromatography was performed by using Quercetin as standard. The Rf value was found to be 0.77 for both standard as well as formulation 3. Almost all of the plant species in the world are thought to have medicinal use, and nearly all of them have excellent antioxidant potential. Increasing oxidative stress has been identified as a major contributing factor to the incidence and development of various diseases, the antioxidant potential of plants has earned a lot of interest (22).In other hand, conventional dosage forms have reported several formulation challenges. Hence, preparation of novel nanoformulation will be benchmarker to overcome such obstacles. Based on the results, phytosome loaded with *Moringa oleifera* Lam extract has showed promising antioxidant potential when it is compared with standard ascorbic acid.

Conclusion

Moringa oleifera Lam is medicinally important plant which has tremendous pharmacological effects such as antidiabetic, anticancer, antimicrobial, antioxidant, anti-inflammatory etc. Due to wide range of phytoconstituents, marketed formulation containing *Moringa oleifera* Lam has gained more attractions as herbal based medicines have no side effects and greater therapeutic potential as compared with synthetic drugs. The major drawback in herbal drug research lies in standardization of phytomedicines. In other hands, traditional dosage forms have several challenges such as poor bioavailability, toxic effects, solubility etc. To overcome these obstacles, novel lipid based nano formulatuions has gained attractions in the effective therapeutic treatment module.

In the present work, attempt has been made to formulate and evaluate phytosome loaded with *Moringa oleifera* Lam extract and its evaluation on antioxidant activities. The present work also aims to develop quality control parameters for an important medicinal plant *Moringa oleifera* Lam. Hence, this tool can be used for further future plant identification and research. The biological activities from prepared phytosome showed promising antioxidant efficiencies. The technique employed for formulation of *Moringa oleifera* Lam will be great tool for the novel nanoformulation development of medicinally important plant. Further, *in vivo* studies should be planned for further conclusions.

Acknowledgement

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Abbreviations

- ICMR-Indian Council of Medical Research
- NITM- National Institute of Traditional Medicine
- RMRC- Regional Medical Research Centre
- DPPH -2, 2-diphenyl-1-picrylhydrazyl
- IC50 -Half maximal inhibitory concentration
- TLC- Thin Layer Chromatography
- PDI- Polydispersability Index
- FTIR- Fourier Transfer Infrared Spectroscopy
- DSC- Differntial Scanning Colorimetry
- Rf value- Retention factor
- UV-Ultra Violet

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