Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2022; 11(3): 250-259
Received: 16-03-2022
Accepted: 24-04-2022

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Formulation and evaluation of polyherbal capsules containing combination of *Terminalia arjuna*, *Chrysanthemum indicum* and *Moringa oleifera*

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Abstract
Natural medicine, especially from herbs, is the source for the research of various novel medicinal compounds. Drugs from herbal origin must be ensured as safe before used as medicine.
Objective: The present work focused on the formulation, development and evaluation of polyherbal capsule containing ethanolic extracts of Bark of *Terminalia arjuna*, flowers of *Chrysanthemum indicum*, leaves of *Moringa oleifera*.
Methods: The ethanolic extract of all three plants were taken for the formulation and combination of all three extract was selected for the formulation of the capsule. The combination of three ethanolic extracts (CTEE) which include Bark of *Terminalia arjuna*, flowers of *Chrysanthemum indicum* and leaves of *Moringa oleifera was* selected as a sample material. The material was weighed, sampled, authenticated and analyzed for their compliance to quality standards. Evaluation of the capsules was done based on different parameters.
Results: Preliminary phytochemical screening of CTEE revealed the presence of major phytochemical groups such as alkaloids, carbohydrates, tannins, steroids and sterols, triterpenoids, saponins and flavonoids. As per the standards, the flow property of the blend to be filled in the capsules should be in good range and was confirmed by the above parameters. Trial batch- 3 showed excellent flow characters and that batch was taken for capsule filling. Physical parameters Moisture content-3.6%±0.22, Uniformity of weight-268 mg ± 4.5mg, Disintegration time-3.32 (min) ± 0.34, pH(1% aqueous solution)-7.33 ± 0.21.
Conclusion: To enhance the acceptability of the herbal medicine by consumers, many of the products have been formulated into conventional dosage forms such as tablets, capsules, suspensions, and powders. CTEE were used in this study to formulate a unit solid dosage form (capsule) to increase the compliances, acceptability and adaptation of the consumers. As it is also very important to estimate the pharmaceutical quality of the Herbal products irrespective of their medicinal content and therapeutic states; so in the present study, the pre-formulation and formulation studies of the formulated capsules. Absorption of drug in the blood is controlled by the availability of drug from solid dosage into the GI fluid. Hence the rate of absorption and availability may be improved by improving the disintegration and the rate of dissolution of drug. Capsule has been successfully formulated.

Keywords: *Terminalia arjuna*, *Chrysanthemum indicum*, *Moringa oleifera*, capsules etc.

Introduction
Hypercholestermia is a life-threatening disorder that develops through elevated lipids content in the blood circulation. Lipids play a vital role in the body's muscle growth, but an abnormal level of fats in the blood highly increases the risk factor for developing coronary heart diseases. Nowadays, cardiovascular diseases are a serious life-threatening epidemic disorder in India [1]. Cardiovascular diseases are responsible for one-third of the total deaths worldwide, and it is believed that cardiovascular diseases will prove to be a leading cause of morbidity and mortality in forthcoming years [2].
Hyperlipidemia is caused by the elevation of total cholesterol, triglycerides, very low density lipoprotein, and low density lipoprotein in plasma. Hyperlipidemia is also caused by a decreased level of high density lipoprotein in blood. Hyperlipidemia with an elevated level of lipoproteins is measured by the initiation and progression of plaque formation in arteries which may causes thrombosis and myocardial infraction [3]. Control and reduction the lipid level is necessary for freedom from coronary artery diseases. However, the drug therapies using niacin, clofibrate, gemfibrozil, atorvastatin, cholestyramine, cholestipol and probucol administered for the treatment of hyperlipidemia may produce an unexpected toxic effect [4]. Probucol especially was withdrawn due to its undesired side-effect of lowering HDL levels

and QT interval prolongation in patients with a previous history of heart diseases. Consequently, herbal rutin and its compound used for the treatment of hyperlipidemia have been approved since it has no undesirable side-effects, its use is economic and easily available [5].

Hyperlipidemia is a disease of lipid metabolism produced by elevation of plasma concentration of the diverse lipid and lipoprotein fractions, which are the source of cardiac disease. It is define as increase serum TC, TG, VLDL, LDL and HDL which are responsible for different complications like: heart attack, coronary artery syndrome, stroke, atherosclerosis, myocardial infarction and pancreatitis. Hyperlipidemia can be either primary or secondary type, the primary syndrome may be treated by hypolipidemic drugs, but secondary induced by diabetes, hypothyroidism or renal lipid nephrosis which treated by treating the original disease moderately than hyperlipidemia. Genetic disorders and lifestyle diet rich in calories, fat, and cholesterol play a vital role to cause dyslipidemia around the world [6]. The main factor which are responsible for hyperlipidemia includes changes in life style habits in which risk factor is mainly poor diet i.e. fat intake greater than 40 percent of total calories, saturated fat ingestion more than 10 percent of total calories; and cholesterol ingestion larger than 300 milligrams per day [7]. For hyperlipidemia large number of synthetic drugs available, not a bit is helpful for all lipoprotein disorders, and each drugs are linked with a number of adverse effects.

Classification of hyperlipidemia

Hyperlipidemia may be classified as either familial (also called primary) caused by definite genetic abnormalities, or acquired (also called secondary) that leads to change in plasma lipid and lipoprotein metabolism.

Familial (primary): -Familial hyperlipidemia is classified as:

Type I: Raised cholesterol with high triglyceride

Type II: High cholesterol with normal level of triglyceride

Type III: High cholesterol and triglycerides

Type IV: Raised triglycerides, and raised uric acid

Type V: Raised triglycerides

Herbal medicine

Herbal Medicine sometimes referred to as Herbalism or Botanical Medicine, is the use of herbs for their therapeutic or medicinal value. An herb is a plant or plant part valued for its medicinal, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substances that act upon the body [8].

Herbal medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization. Much of the medicinal use of plants seems to have been developed through observations of wild animals, and by trial and error. As time went on, each tribe added the medicinal power of herbs in their area to its knowledgebase. They methodically collected information on herbs and developed well-defined herbal pharmacopoeias. Indeed, well into the 20th century much of the pharmacopoeia of scientific medicine was derived from the herbal lore of native peoples. Many drugs commonly used today are of herbal origin. Indeed, about 25 % of the prescription drugs dispensed in the United States contain at least one active ingredient derived from plant material. Some are made from plant extracts; others are synthesized to mimic a natural plant compound [9].

Experimental work

Chemicals and reagents

Ethanol 99.9% was procured from LOBA Chemicals, Mumbai. Ethylene di-aminetetraacetic acid (EDTA) was procured from Thermo Fisher Sci- entific India Pvt. Ltd., (Mumbai, India). All the sol- vents used were of high purity and HPLC grade. All other chemicals and reagents used in the whole study were of analytical grade.

Pharmacognostic evaluation of plant materials

Collection and authentication of plant material

The Bark of *Terminalia arjuna*, flowers of *Chrysanthemum indicum* and leaves of *Moringa oleifera* was collected locally in the Thane district. The plant materials were then authenticated from Ideal College of Pharmacy and Research, Kalyan.

Preparation of extracts

The collected plant material (Bark of *Terminalia arjuna*, flowers of *Chrysanthemum indicum* and leaves of *Moringa oleifera)* (500 g) each was gently washed by using distilled water to remove the impurities. The collected materials were shade dried in the laboratory under room temperature (24 ± 2 °C) for 3–4 weeks. After complete drying, the dried plant material was and pulverized by using a mechanical grinder followed by sieving to obtain a coarse powder. The powdered plant material was then extracted with distilled water and ethanol (99.9%) using reflux technique separately. Extracts were concentrated by vacuum distillation and then dried in open air to produce the respective extracts. The crude aqueous and ethanol extracts obtained was stored at 4 °C before analysis. The percentage yield of the extract was calculated by the following formula.

Percentage yield = (Weight of dry crude extract obtained/weight of plant material before extraction) X 100

The weight in gram was used to calculate the percentage yield. The calculated percentage yield was as follows;

No.	Extract	Percentage yield
1	Aqueous extract of Bark of *Terminalia arjuna*	7.24%
2	Aqueous extract flowers of *Chrysanthemum indicum*	6.34%
3	Aqueous extract leaves of *Moringa oleifera*	4.48%
4	Ethanol extract of Bark of *Terminalia arjuna*	8.54%
5	Ethanol extract flowers of *Chrysanthemum indicum*	5.35%
6	Ethanol extract leaves of *Moringa oleifera*	7.78%

Phytochemical screening

The Phytochemical screening was done by the standard procedure as depicted in Table:

Table 1: Preliminary Phytochemical Screening

Chemical constituents	Chemical test
Proteins	Biuret test
Carbohydrates	Molish test
	Fehling's test
Alkaloids	Dragendorff's test
	Mayer's test
Steroids	Salkowaski test
	Liebermann-burchard test
Triterpene	Vanillin-sulphuric acid test
Tannins	Ferric chloride test
	Dilute nitric acid test
Glycosides	Keller-killani test
Flavonoids	Shinoda test
	Lead acetate test
Saponins	Foam formation test
Amino acids	Ninhydrin test

Preformulation studies

To formulate any dosage forms, it is essential that fundamental physical and chemical properties of the drug powder are to be determined [66, 67].

Definition

Preformulation involves the application of biopharmaceutical principles to the physicochemical parameters of drug substance are characterized with the goal of designing Optimum drug delivery system. Before beginning the Preformulation programs the preformulation scientist must consider the following factors

- The amount of drug available.
- The physicochemical properties of the drug already known.
- Therapeutic category and anticipated dose of compound.
- The nature of information, a formulation should have or would like to have

Selection of excipients

For the formulation of capsules in addition to the active ingredients, excipients like diluents (filler), binder, disintegrating agent, lubricant and preservatives are required. The choice of excipients was made keeping in mind the current Food and Drugs Administration (FDA) regulations. [68].

Diluents

Diluents/Fillers are added where the quantity of active ingredient is less (or) difficult to filling. Common tablet/capsule filler include Lactose, Dicalcium phosphate, microcrystalline cellulose, etc.

Lubricants

They reduce friction during the filling process. In addition, they aid in preventing adherence of capsule material. Magnesium Stearate, Stearic acid, Hydrogenised vegetable oils and talc are commonly used lubricants.

Glidants

It is used to improve flow of the powder materials by reducing the friction between the particles. The most effective glidants are the Colloidal silicon dioxide, Talc and Starch.

Preservatives

The preservatives are added to herbal formulation to prevent contamination, deterioration and spoilage by bacteria, fungal and other microorganisms. The most effective preservatives are the sodium methyl paraben, sodium propyl paraben, sodium benzoate and bronopol.

Selection of excipients in the formulation are given below

- Talc - Glident/Lubricant
- Microcrystalline cellulose - Diluent/Disintegerant
- Starch - Binder/Disintegerant
- Colloidal sillicon dioxide - Glident
- Magnesium stearate - Lubricant
- Bronopol - Preservative
- sodium methyl Paraben - Preservative

Preparation of formulation

- The dry CTEE (Combination of Three Ethanolic Extracts) were dried in tray drier at 60 °c for 20 minutes. All excipients used in this formulation except preservatives were dried separately in tray drier at 100oc for 30 minutes. All active ingredients were weighed according to the formula, mixed and lubricated with magnesium stearate followed by diluents and preservatives were mixed well. The mixture was blended thoroughly for 30 minutes. Then the powder was transferred to the polythene bags and labelled for further studies [115].

Development of formulation-trial batches

Three trial batches were formulated by varying the composition of the excipients proportions for excellent flow properties.

Table 2: Development of formulation

No.	Materials	Trial-1 (g)	Trial-2 (g)	Trial-3 (g)
1	CTEE	20	20	20
2	Talc	1.7	1.8	2.5
3	Mcc	0.8	0.9	1
4	Starch	2	2.5	2.6
5	Magnesium stearate	0.8	1	1.5
6	Colloidal sillicon dioxide	0.25	0.28	0.3
7	Bronopol	0.15	0.15	0.15
8	Sodium methyl paraben	0.15	0.15	0.15

Evaluation of blended powder
The blended powder of all trial batches were analysed for its flow characteristics like bulk density, tap density, compressibility index, Hausner's ratio and angle of repose.

Bulk density and tap density and Carr's index
A weighed quantity w of (15g) powdered material was taken in a 50 ml measuring cylinder. And recorded the initial volume (vo) tapped the contents and recorded the powdered volumes after 50 taps (v50).
The formula for fluff density and Tapped density is as follow
Fluff density = w/vo g/cc
Tapped density = w/vo 50 g/cc
The formula for Carr's index is as follow

Carr's index = {Tapped density- Fluff density/ Tapped density} X 100
Value for Carr's index below 15 indicate excellent flowing material and value over 20-30 suggested poor flowing material.

Angle of repose
A funnel was fixed at a particular height (1.5, 2.5, 3.5 cm) on a burette stand. A white paper was placed below the funnel on the table. The powdered drug passed slowly through the funnel until it forms a pile. The radius of the pile was noted down. Angle of repose of the powder material was calculated by using the formula:

Tan θ = h/r,θ = tan (h/r) where, h = height of the pile, r = radius.

Values for angle of repose < 30o usually indicate a free flowing material and angle > 400 suggest a poor flowing material.

Hauser's ratio
The basic procedure is to measure the unsettled apparent volume, V_0 and the final tap volume V_f , of the powder tapping the material until no further volume changes occur.
The Hausner's ratio was calculated as follows:
Hausner's ratio = V_0/V_f

Hausner's ratio between 1.00 to 1.11 shows excellent flow and value more than1.60 shows very poor flow.

Formulation of capsules [77-82]
From the 3 trial batches one optimized batch is selected for formulation based on above results. Trial batch 3 was found to be the perfect batch and it was selected for the consideration of further evaluation.

Table 3: Final batch composition -250 mg/capsule

No	Ingredients	Quantity in mg
1.	CTEE	200
2.	Micro crystalline cellulose	12.5
3.	Starch	7.4 -10
4.	Sodium methyl paraben	0.25
5.	Talc	25
6.	Colloidal sillicon dioxide	3.7
7.	Magnesium stearate	0.85
8.	X-Bronopol	0.25

Capsules are small dosage form in which one or more medicinal and inert ingredients are enclosed in a small shell usually made of gelatin.

Capsule Size and Selection of Filling Method
- The formulated granules were filled in "1" size capsules to an average net content t weight of 270 mg.
- The capsules were then de dusted, transferred into polybags, labelled and the Samples were evaluated as per the testing requirements.
- A hand operated gelatin capsule filling machine was used in this study for encapsulation of capsules.
- From the final trial, samples were taken for accelerated stability studies as per the testing requirements.

Fig 2: Herbal capsule

Standardisation of herbal capsules [70-75]
The developed herbal capsules were standardized for its description, uniformity of weight, disintegration time, moisture content, physicochemical parameters, phytochemical studies, fluorescence analysis. Standardization were carried out as per Indian pharmacopoeia procedures.
Following Quality control parameters were evaluated

1. Description
The general appearance of a capsule, its visual identity and overall "elegance" is essential for consumer acceptance. The color, shape, odor and surface texture are all noted for the capsules prepared.

2. Uniformity of weight
20 individual units were selected at random and their content was weighed and their Average weight was calculated. Not more than two of the individual weights deviate from the average weight by more than the percentage shown in the table

Table 4: Acceptance Criteria I.P Limit

Dosage form	Average limit	Deviation
capsules	< 300 mg	10%
	>300mg	7.5%

3. Disintegration test
Disintegration test was performed using the digital microprocessor based disintegration test apparatus. One capsule was introduced into each tube and added a disc to

each tube. The assembly was suspended in the water in a 1000 ml beaker. The volume of water was such that the wire mesh at its highest point is at least 25 mm below the surface of water, and at its lower point was at least 25 mm above the bottom of the beaker. The apparatus was operated and maintained the temperature at 37±2 °C. (Indian Pharmacopoeia, 2010).

4. Determination of moisture content

The loss on drying test is important when the herbal substance is known to be Hygroscopic. An excess of water in medicinal plant materials will encourage Microbial growth, the presence of fungi, insects deterioration. In modern Pharmaceutical technology, the water content provides information concerning the Shelf life and quality of the drugs.

Moisture content (%) = {Final weight of the sample/Initial weight of the sample} ×100

5. pH

1 g of capsule powder was taken and dissolved in 100 ml demineralized water. The pH value of the solution was determined by means of a digital pH meter. The pH meter was calibrated using buffers of 4, 9 and 7 pH. The electrodes were immersed in the test solution and pH was measured.

Accelerated stability studies of the capsules

Stability is defined as the extent to which a product retains, within specified limits and throughout its period of storage and use (i.e., its shelf-life) the same properties and characteristics that it possessed at the time of its manufacture.

6. Accelerated testing

Studies designed to increase the rate of chemical degradation or physical change of a drug substance or drug product by using exaggerated storage conditions as per of the formula stability studies. Date from the studies, in addition to long tern stability studies, can be used to assess longer term chemical effect at non acceleration and to evaluate the effect of short – term excursions outside the label store conditions such a might occurs during shipping. Result from accelerated testing studies are not always predictive of physical changes.

Conditions of Stability studies

- Accelerated condition of 40 °C ± 2 °C/75% RH ± 5% RH
- Long term condition of 25 °C ± 2 °C/60% RH ± 5 % RH
- Long term / intermediate condition of 30 °C ± 2 °C/75 % RH ± 5% RH

The ICH Harmonized Tripartite Guideline provides a general indication on the requirements for stability testing of new drug substances and products. The main thrust of the stability guideline centers on criteria for setting up stability protocols.

6. Climatic zones

The four zones in the world that are distinguished by their characteristic prevalent annual climatic.

Table 5: Climatic zones and derived storage conditions

Zone	Condition	Temperature	Humidity
Zone 1	Temperature	21ºc	45% RH
Zone 2	Sub-tropical with possible Humidity	25ºc	60% RH
Zone 3	Hot/ dry	30ºc	35% RH
Zone 4	Hot / humid	40ºc	70% RH

Accelerated stability condition : Accelerated stability study were carried out of storage condition at 40 °C ± 2 °C of humidity 70% RH for 3 month(time period covered).

Result and Discussion

Preliminary phytochemical screening

Preliminary phytochemical screening of CTEE revealed the presence of major phytochemical groups such as alkaloids, carbohydrates, tannins, steroids and sterols, triterpenoids, saponins and flavonoids as shown in Table.

Table 6: Preliminary phytochemical screening of three selected plant

Chemical constituent	Chemical test	Bark of *Terminalia arjuna*	Flowers of *Chrysanthemum indicum*	Leaves of *Moringa oleifera*
		Ethanolic extract	Ethanolic extract	Ethanolic extract
Proteins	Biuret test	+	+	+
Carbohydrate	Molish test	+	+	+
	Fehling's test	+	+	+
Alkaloid	Dragendorff's test	-	-	+
	Mayer's test	-	-	+
Steroids	Salkowaski test	+	-	-
	Liebermann-burchard test	+	-	+
Triterpene	Vanillin-sulphuric acid test	+	-	+
Tannin	Ferric chloride test	-	+	+
	Dilute nitric acid test	-	+	+
Glycoside	Keller-killani test	+	+	-
Flavonoid	Shinoda test	-	+	+
	Lead acetate test	-	+	+
Saponins	Foam formation test	-	-	+
Amino acids	Ninhydrin test	+	-	-

Phytochemical study reveals the presence of some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids, flavonoids, and saponins.

Result of polyherbal formulation capsule

The combination of three ethanolic extracts (CTEE) which include Bark of *Terminalia arjuna*, flowers of *Chrysanthemum indicum* and leaves of *Moringa oleifera was* selected as a sample material. The material was weighed,

sampled, authenticated and analyzed for their compliance to quality standards as established by WHO guidelines, pharmacopoeial and other standard reference books.

Preformulation Studies

Three trial batches of the herbal formulation were prepared and tested for Preformulation parameters like bulk density, tap density, Carr's index, Hausner's ratio and Angle of repose. The results observed is shown in table.

Table 7: Final batch composition -250 mg/capsule

No	Ingredients	Quantity in mg
1.	**CTEE**	200
2.	Micro crystalline cellulose	12.5
3.	Starch	7.4 -10
4.	Sodium methyl paraben	0.25
5.	Talc	25
6.	Colloidal sillicon dioxide	3.7
7.	Magnesium stearate	0.85

Table 8: Evaluation of in process parameters

Parameters	Trial-1	Trial-2	Trial-3
Bulk density (g/cm)	0.42±0.01	0.38±0.05	0.35±0.04
Tap density (g/cm)	0.45±0.03	0.47±0.01	0.50±0.04
Compressibility index(%w/w)	26.83±0.66	23.26±2.54	13.06±1.12
Hausner ratio	1.35±0.15	1.22±0.02	1.13±0.01
Angle of repose	40.42±2.57	39.36±2.67	34.66±0.18

All values are expressed as standard mean deviation ±, where n=3

Table 9: Evaluation of in process parameters

Parameters	Trial-1	Trial-2	Trial-3
Flow property	Normal	Fair	Perfect
Filling	Uniform	Uniform	Uniform
Weight	Not uniform	Not Uniform	Uniform
Moisture content	Satisfied	Satisfied	Perfect
Disintegration time	Within the limit	Within the limit	Perfect

As per the standards, the flow property of the blend to be filled in the capsules should be in good range and was confirmed by the above parameters. Trial batch- 3 showed excellent flow characters and that batch was taken for capsule filling.

The trial 3 flow properties were Excellent and all parameter were within the Specified limits. So, third trial was chosen for further studies.

Standardisation of finished formulation

The final batch was tested for organoleptic characters, physical and physico chemical parameters. The results observed are shown in table

Table 10: Organoleptic characters

Name of test	Observations
Description	Pale brown powder contained in purple cap/ transparent body "1" size capsule
Coluor	Reddish brown powder
Odour	Characteristic odour
Taste	Bitter

Table 11: Physical parameters

Name of the test	Observations
Moisture content	3.6 % ± 0.22
Uniformity of weight	268 mg ± 4.5mg
Disintegration time	3.32 (min) ± 0.34
pH(1% aqueous solution)	7.33 ± 0.21

Results (n-=3) are reported as mean ± standard deviation.

- 1% aqueous solution of herbal formulation showed acidic pH.
- The average weight of the capsules was calculated as per I.P and the obtained value was with in the limit (±10%).
- Sample were taken randomly (3times) to specify quantity, the moisture content was calculated as per trail and error by KFR titration method. The result were given in the above table.
- Disintegration time of the herbal capsule was performed as per I.P and the obtained value showed that the capsule will be disintegrated within the prescribed time for the absorption.
- The uniformity of weight of the capsules was calculated as per the I.P and obtained value was within limit (±7.5).
- The formulated herbal capsule weight were the lower limit is noted as 248 mg and the upper limit is noted as 287mg.

The rate and extent of absorption of a drug into the bloodstream is an important quality characteristic of a dosage form. *In vivo* bioavailability and *in vitro* dissolution studies are important in the development and ultimately in the quality control of a dosage form. Formulation studies involve developing a preparation of the drug which is both stable and acceptable to the patient. For orally taken drugs, this usually involves incorporating the drug into a tablet or a capsule. The medicine derived from plants can be used more conveniently and safely in various diseased conditions, if used in proper portions and combination. To enhance the acceptability of the herbal medicine by consumers, many of the products have been formulated into conventional dosage forms such as tablets, capsules, suspensions, and powders. CTEE were used in this study to formulate a unit solid dosage form (capsule) to increase the compliances, acceptability and adaptation of the consumers.

Conclusion

As it is also very important to estimate the pharmaceutical quality of the Herbal products irrespective of their medicinal content and therapeutic states; so in the present study, the pre-formulation and formulation studies of the formulated capsules. Absorption of drug in the blood is controlled by the availability of drug from solid dosage into the GI fluid. Hence the rate of absorption and availability may be improved by improving the disintegration and the rate of dissolution of drug. Capsule has been successfully formulated. The formulated Dosage forms met the pharmacopeia criteria for quality Assessment and can be used as suitable alternatives in the Management and treatment of hyperlipidemia.

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