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Research Article

ANTHELMINTIC SHANKHPUSHPI PELLETS: TASTE MASKING

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ABSTRACT

Shankhpushpi is a highly potent anthelmintic drug having bitter taste. In the formulation for pediatric & geriatric patients the main challenge to the formulator is to mask the taste of obnoxious and bitter drugs without loss of optimal therapeutic activity of the formulation. The main objective of present study was the in vitro evaluation of the taste masking efficiency and anthelmintic activity of shankhpushpi formulation prepared by extrusion-spheronization. Pellets containing high shankhpushpi loadings in Eudragit L-100-55 were prepared by extruder and spheronizer. The taste masking of processed formulation was evaluated in vitro by dissolution method and chromatographic technique, while anthelmintic effect was evaluated by using six adult Indian earthworms and cattle worms. The result of present study indicated that Shankhpushpi for few minutes began the paralysis of earthworm followed by death at the end of 375.5 minutes. Thus, the present study demonstrated that formulated pellets of Shankhpushpi has potent anthelmintic activity and has commercial significance.

Keywords: Pellets, Anthelmintic, Taste masking, Extrusion-Spheronization.

INTRODUCTION

Nature has provided a complete store house of remedies to cure all ailments of mankind and its related diseases. The human being appears to be affected with more diseases than other animal species. There can be little doubt that is sought out to alleviate human suffering from injury and diseases by taking advantage of plants of the surroundings. In the past, almost all the medicines used were extracted from the plants and the plant being man's chemist for ages.[1]

In the recent years, the importance of herbal drugs in medicine has tremendously increased because of their fewer side effects. Consequently, the demand for the herbal formulation is increasing day by day. The phytochemical constituents and their standardization are accelerated with the development of instrumental analysis and this field becomes important and new for investigation.[2]

Infection with helminths are among the most widespread infections in humans and other domestic animals affecting a large number of world populations. The majority of these infections due to worms are generally restricted mainly to the tropical regions and the occurrence is accelerated due to unhygienic lifestyle and poverty also resulting in the development of symptoms like anaemia, eosinophilia and pneumonia.[2] These infections can affect most populations in endemic areas with major economic and social consequences. Because of limited availability and affordability of modern medicines most of the world's population depends to a greater extent on traditional medical remedies. The traditional medicines hold a great promise as source of easily available effective anthelmintic agents to the people, particularly in tropical developing countries, including India. Ideally an anthelmintic agent should have broad spectrum of action, high percentage of cure with a single dose, free from toxicity to the host and should cost effective. The origin of many effective drugs is found in the traditional medicine practices and in view of this several researchers have undertaken studies to evaluate medicinal plants for their proclaimed anthelmintic efficacy.[3] Natural anthelmintic includes- Moringaoleifera[4], Neem[5], Papaya seeds[6], Shankhpushpi[7], Wormwood[8], Clove[9], Garlic[1], Kalonji[10] and any others.

Shankhpushpi shows better anthelmintic activity. Shankhpushpi is a perennial herb that seems like morning glory. Its branches are spread on the ground and can be more than 30 cm long. The flowers are blue in colour (5mm) and the leaves, which are elliptic in shape (2mm), are located at alternate positions with branches or flowers. Known as Aloe weed in English, the herb is commonly found in India, especially in the state of Bihar. All the parts of the herb are known to possess therapeutic benefits. It is believed to be the only herb that is capable of enhancing all the aspects related to brain power, such as learning, memory and the ability to recall. However, its popularity stems from its ability to treat insomnia and helmenthis effectively.[11] But its bitter taste leads to poor patient compliance in pediatric and geriatric population. Thus taste masking of these bitter drugs is one of the challenging task in front of manufacturing companies.

The taste masking of bitter APIs is a major challenge especially for the development of oral formulations in pharmaceutical industry. Several approaches have been reported which involve fluidized-bed coating, supercritical fluids and coacervation approaches where effective taste masking is achieved by applying polymeric coating layer to create a physical barrier around the drug.[12] Other alternatives involve the use of complexing agents (cylcodextrins, ion exchange resins) through the formation of inclusion complexes or resonates.[13] Recently, taste masking approaches have employed taste suppressants molecules by blocking the gap junction channels and hemi channels and thus suppressing the drugs taste.[14,15] However, there is an enormous need for more robust, cost effective and easy to scale-up taste masking technologies. Extrusion spheronization is a continuous, one step process that can be used for the preparation of taste masked pellets.

Extrusion Spheronization has been employed as a novel technique for the formulation of oral solid dosage forms in pharmaceutical industries in the last decade. It was initially used in food and plastic industry but has attracted significant interest in pharmaceutical manufacturing for the development of robust formulations. Extrusion Spheronization can be used to develop various formulations such as sustained release Pellets.[16,17] It has been also introduced for taste

masking purposes of bitter APIs by involving the use of taste masking polymers that prevent bitter drugs from coming in contact with the patient's taste buds.[18]

Pellets are agglomerates of fine powders or granules of bulk drugs and excipients. They consist of small, free flowing spherical or semispherical solid units typically from about 0.5-2mm.[19]

These are intended usually for oral administration. These are spheres of varying diameter depending on the application and the wish of the producer. Pellets are prepared using different technologies such as layering of the drug solution, suspension or powder on the inactive cores, extrusion, spheronization and agglomeration in roto-granulators or roto processors, compression, spray drying and spray congealing.[20]

MATERIALS AND METHODS

The plant material was purchased from Shivshankar Ayurvedic Agency Nagpur, Maharashtra, India. Eudragit L-100-55 was purchased from Vikram Drugcoat. MCC PH 102 was purchased from Alka scientific Company. Triethyl citrate and PVP K-30 was purchased from Himedia Laboratories Pvt. Ltd. Mumbai, Maharashtra, India. All materials were used as received.

Procedure for extraction

Aerial parts of Shankhpushpi were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction by maceration method. In the extraction process, the 250g of powdered material of the shankhpushpi was macerated with 500 ml of distilled water for 15 days. After complete extraction, the macerated material was filtered through the muslin cloth and concentrated on water bath.

Preliminary Phytochemical Evaluation

Determination of Physical Constants[21,22]

Loss on Drying (LOD)

Loss on drying is the loss of mass expressed as percent w/w. The test for loss on drying determines both water and volatile matter in the crude drug. Moisture is an inevitable component of crude drug, which must be determined as far as possible.
An accurately weighed quantity of about 5 g of drug was taken in a tared porcelain dish. The porcelain dish was kept open in oven and the sample was dried at a temperature 110 ^{0}C for 2 hr until a constant weight was recorded. Then it was cooled in desiccator to room temperature, weighed and recorded. Percent loss on drying was calculated using formula.

% LOD = Loss in weight of the sample / Weight of the sample × 100

Ash Value

Ash value is helpful in determining the quality and purity of a crude drug. The objective of ashing herbal drugs is to remove all traces of organic matter, which may otherwise interfere in an analytical determination.
Accurately weighed 2 to 3 g of the drug in a tared silica crucible, incinerated at a temperature until free from carbon, cooled and weighed. Calculate the percentage of ash with reference to air-dried drug using following formula:

% Total ash value = Weight of total ash / Weight of crude drug taken × 100

Determination of Total Bitters

Weight accurately 3 g extract in a beaker and dissolved in 30 ml methanol by warming on water bath, transfer the contents into 100 ml round bottom flask, wash the beaker with 5 – 10 ml methanol and transfer to the same flask. Reflux for half an hour and repeat the process till bitterness persists in residue. Pool extraction washings and distilled off alcohol under reduced pressure. Take up residue repeatedly with 25, 15and 10 ml of hot water. Shake the combined aqueous extract with 20, 10 and 10 ml of n-hexane and discard. Aqueous extract is then repeatedly extracted with 25, 20, 15 and 15 ml of ethyl acetate. Pool ethyl acetate washing, evaporated to dryness to constant weight.

Total Bitter % = Weight of residue / Weight of the sample × 100

Qualitative Chemical Tests[23]

Tests for Flavonoids
(i) Shinoda Test: To the extract, added 5 ml of 95 % ethanol, few drops concentrated HCl and 0.5 g magnesium turnings. Pink colour was observed.
(ii) Ferric Chloride Test*:* To a test solution, added few drops of ferric chloride solution observed for intense green colour.

Tests for Triterpenoids
Libermann-Burchard Test**:** Mixed 3 ml extract with chloroform. Added 1-2 ml acetic anhydride and 2 drops concentrated H_2SO_4 from the side of the test tube observed for first red, then blue and finally green colour.

Tests for Tannins
(i) Ferric Chloride Test**:** To2-3 ml of extract, added few drops of 5 % $FeCl_3$, observed for deep blue-black colour.
(ii) Acetic Acid Test**:** To 2 ml of extract, added acetic acid solution, observed red colour solution.

Tests for Amino Acids
Ninhydrine Test (General Test): 3 ml of extract and 5 % Ninhydrin solution were heated in boiling water bath for 10 min. Observed for purple or bluish colour.

Test for Alkaloids
Extracts were dissolved individually in dilute Hydrochloric acid and filtered.
(i) Mayer's Test**:** Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.
(ii) Wagner's Test**:** Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.
(iii) Dragendroff's Test**:** Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.
(iv) Hager's Test**:** Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

Test for Carbohydrates
Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.
(i) Molisch's Test*:* Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.
(ii) Benedict's Test**:** Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

(iii) Fehling's Test**:** Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Test for Glycosides
Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.
(i) Modified Borntrager's Test**:** Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.
(ii) Legal's Test**:** Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

Test for Saponins
(i) Froth Test**:** Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.
(ii) Foam Test**:** 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Test for Phytosterols
(i) Salkowski's Test**:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.
(ii) Libermann Burchard's test**:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Test for Phenols
Ferric Chloride Test**:** Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Test for Alkaloids
(i) Wagner's reagent**:** A fraction of extract was treated with 3-5drops of Wagner's reagent [1.27g of iodine and 2g of potassium iodide in 100ml of water] and observed for the formation of reddish brown precipitate (or colouration).

Test for Phlobatannins
Precipitate test: Deposition of a red precipitate when 2mls of extract was boiled with 1ml of 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

Test for Quinones
A small amount of extract was treated with concentrated HCL and observed for the formation of yellow precipitate (or colouration).

Test for Oxalate
To 3ml portion of extracts were added a few drops of ethanoic acid glacial. A greenish black colouration indicates presence of oxalates.

UV spectrophotometric study on the drug preparing the tincture[24]

Appropriate quantity of extract was dissolved in 100 ml distilled water and the resultant tincture was studied under UV-Visible Spectrophotometer.

Anthelmintic Activity of extracts

This experiment was conducted to evaluate the anthelmintic activity of Aqueous extract of shankhpushpi as follows.

Experimental Animals

Adult earthworms (*Pheretima posthuma)* and Cattle worms were used to evaluate anthelmintic activity in-*vitro*. Earthworms were collected from moist soil and washed with normal water to remove all faecal matter were used for the anthelmintic study. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol.[25, 26]

Anthelmintic Activity

The anthelmintic assay was performed in-vitro using adult earthworms (*Pheretima posthuma*) as it is having anatomical and physiological resemblance with the intestinal round worm parasites of human beings for preliminary evaluation of anthelmintic activity.[27] Groups of approximately equal sized earthworms consisting of six worms in each group were made. Each group was treated with one of the following –

Group 1 :	Control	Distilled Water
Group 2 :	Albendazole (Reference)	400 mg/25ml
Group 3 :	Aq. Shankhpushpi extract	0.05 mg/25ml
Group 4 :	Aq. Shankhpushpi extract	0.1 ml/25ml
Group 5 :	Aq. Shankhpushpi extract	0.2 ml/25ml
Group 6 :	Aq. Shankhpushpi extract	0.25 ml/25ml
Group 6 :	Aq. Shankhpushpi extract	0.3 ml/25ml
Group 11 :	Marketed Albendazole Tablet	400 mg/25ml

Test samples of the extract were prepared in distilled water and worms of approximately equal size were placed in each nine cm petridish containing 25 ml of above test solution of extract. All the test solution and standard drug solution were prepared freshly before starting the experiments.

Observations were made for the time taken for paralysis of individual worm. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50^0 C).

Pellet Formulations of Shankhpushpi Extract

Oral formulations are one of the most widely used and accepted dosage form in medicine especially in herbal formulations. Considering its convenience and ability to mask unpleasant taste and odour of herbal extracts, pellet formulation is selected. Shankhpushpi exhibiting significant pharmacological Anthelmintic activity were selected and formulated into oral pellets.

Pellets were prepared by extrusion spheronization method. In pellet formulation Triethyl Citrate used as plasticizer, Polyvinyl Pyrrolidone (PVP) as binder and Eudragit L 100-55 as Coating Polymer.[28]

Taste Mask Formulations

In the initial formulation study the pellets containing Eudragit and MCC in different ratio was prepared (SP/ 01 to SP/03) as given in Table 1.

Table 1: Formulation composition of anthelmintic herbal pellets by Taste Masking method with different ratios

	Pellet composition (% w/w)		
	SP/I-01	**SP/I-02**	**SP/I-03**
Drug (Extract)	25 g of pellets contain 12.5 ml liquid Extract		
MCC	50 %	40 %	45 %
Eudragit L-100-55	44 %	54 %	49 %
Triethyl Citrate	11 % of Eudragit	11 % of Eudragit	11 % of Eudragit
PVP K-30	2 %	2 %	2 %
Distilled Water	q. s.	q. s.	q. s.

Amongst the various ratio, the formulation SP/I- 02 was found to be suitable for further studies as it satisfy the properties of good pellets. SP/I-02 formulation was considered constant and the Shankhpushpi extract load was varied to prepare formulations SP/I-02 a to SP/I-02 d (Table 2).

Table 2: Formulation composition of anthelmintic herbal pellets by Taste Masking method with different drug loading

	Pellet composition with different drug (Extract) loading (% v/w)			
	SP/02 a	**SP/02 b**	**SP/02 c**	**SP/02 d**
Drug (Extract)	5 ml	10 ml	12.5 ml	15 ml
MCC	40 %	40 %	40 %	40 %
Eudragit L-100-55	54 %	54 %	54 %	54 %
Triethyl Citrate	11 % of Eudragit	11 % of Eudragit	11 % of Eudragit	11 % of Eudragit
PVP K-30	2 %	2 %	2 %	2 %
Distilled Water	q. s.	q. s.	q. s.	q. s.

The extract was adsorbed in the MCC for 10 min. Then extract adsorbed MCC and Eudragit L 100-55 were mixed in a mortar pestle for 5 min. Triethyl citrate was added as a plasticizer, and the resultant mixture was triturated in a mortar for 5 min. Polyvinylpyrrolidone (PVP K-30) as a binder were added and mixed for 10 min in a mortar pestle. This mixture was granulated with distilled water in a mortar. To ensure uniform distribution of water during wetting phase, the material was repeatedly scrapped from the mixing mortar walls and later extruded at a screw speed of 40 rpm using a single screw extruder Fig. 6.1 (a) (Anish Pharma Equipment Pvt. Ltd.) equipped with a dome-shaped extrusion screen. The extrudates immediately were transferred onto a rotating plate in the spheronizer Fig. 6.1 (b) (Anish Pharma Equipment Pvt. Ltd.), which consisted of a stationary vertical cylinder with a friction plate. Spheronization was carried out for 5 min at 550 rpm, followed by 15 min at 850 rpm. During the first 5 min period, 5% w/w of the total batch-size MCC PH 101 was sprinkled over the rotating extrudates to prevent the pellets from sticking. Pellets obtained were dried on tray dryer at 50 ^{0}C for 2 hr.

Characterisation of Pellets[29]

In order to meet the requirements of pellet yield, size distribution, surface area, shape, surface roughness, density and friability, including the reproducibility of morphologic properties of the pellets, pellets were tested.

Pellet yield

The prepared pellets (20 g) were sieved for 5 min on sieve shaker equipped with series of sieves of pore opening as 1400, 1000, 710, 500 and 250 µm sieves (Sieve No. 12, 16, 22, 30 and 60 respectively). The pellet yield was calculated based on the pellet fraction between 710 and 1400 µm and presented as a percentage of the total pellet weight. This size fraction was used for all further measurements.

Size Analysis

The most common and widely used method for determination of size is sieve analysis. The reasons for its extensive use are simplicity, low costs, low time consuming. Sieving, using sieve shaker is one of the fundamental methods for determining the size distribution of pellets. In this method test sieves ranging from sieve no. 12 to 60 are arranged in descending order. A 20 g quantity of the pellets is placed on the top sieve and the set-up is shaken for 5 min. The weight of material retained on each sieve is determined.The modal class fraction was the size fraction obtained from sieving with the highest weight of pellets. The average diameter is calculated using the equation:

$$\text{Average Diameter} = \sum (\%\ \text{retained}) \times (\text{Mean Aperture}) / 100$$

Shape Analysis

At least 20 pellets from each batch were randomly selected for shape analysis from fraction obtained after size analysis by sieving. The pellets were mounted on a surface of motic microscope, and the images of the pellets were captured. The area of the images and the maximum and minimum radii were calculated, and from these the various shape factors were calculated.

Densities of Pellets

An accurately weighed quantity (5g) of the pellets (W), was carefully poured into the graduated cylinder and the bulk volume (Vo) was measured. Then the graduated cylinder was closed with lid, set into the density determination apparatus. The density apparatus was set for 100 tabs and after that, the volume (Vf) was measured and operation was continued till the two consecutive readings were equal. The bulk density, and tapped density were calculated using the formulae**.**

$$\text{Bulk density} = \text{Weight} / \text{Bulk volume}$$

$$\text{Tapped density} = \text{Weight} / \text{Tapped volume}$$

Compressibility Index and Hausner's ratio

In recent years the compressibility index and the closely related Hausner's ratio have become the simple, fast, and popular methods of predicting pellets flow characteristics. Both the compressibility index

and the Hausner's ratio were determined by using bulk density and the tapped density of pellets.

Carr's Index = Tapped density – Bulk density / Tapped density × 100

Hausner's Ratio = Tapped density / Bulk density

Flow Rate and Angle of Repose

The flow rate and angle of repose has been used to characterize the flow properties of solids. Angle of repose is a characteristic related to inter particulate friction or resistance to movement between particles. This is the maximum angle possible between surface of pile of pellets or granules and the horizontal plane.

Flow rate (g/sec) = Weight of pellets / Time

$\Theta = \text{Tan}^{-1}\ h/r$

Where, **θ** = angle of repose, **h** = height of pile, **r** = radius of pile

A funnel was fixed at a height approximately of 2-4 cm over the platform. The loose pellets (10 g) was filled in a funnel and passed along the wall of funnel, till the cone of the pellets formed. The angle of repose was determined by measuring the height of the cone of pellets and radius of the heap of pellets.

Pellet Friability Test

10g of pellets (Fs) was placed in friability test apparatus together with 20 glass beads. The sample was subjected to falling shocks for 4 minutes at a rotational speed of 25 rpm and fines collected by sieving through 250 µm mesh. The weight difference was obtained by weighing the pellets retained above 250µm (Fa) and compared to the initial weight of the sample and percentage of friability determined using the following equation:

Friability (%) = {(Fs - Fa)/Fs} × 100

Pellet Disintegration Test

The pellet disintegration in water was evaluated by a tablet disintegration test apparatus. 100 mg pellets were placed along with a plastic disc in each tube and they were inserted in the disintegration test apparatus maintained at 37°C ± 1°C. Disintegration test was carried out three times for each formulation, and results were expressed with the standard deviations.

Evaluation of Taste Masking Of Shankhpushpi Pellets

Gustatory Sensation Test for Taste Masking

The gustatory sensation test was carried out in six healthy male volunteers. The taste masking efficiency of the used methods were analysed by gustatory sensation test where the level of taste masking was rated using a Hedonic Rating Scale for taste perception.

Table 3: Hedonic Rating Scale for Taste Perception

Rating	1	2	3	4	5	6	7
Description	Extremely Poor	Very Poor	Poor	Fair	Good	Very Good	Excellent

Taste masking test using Dissolution Method

The pellet formulations SP/I-02 c and SP-09 were subjected to micro-dissolution method. The modified apparatus shown in Fig. 6.3 by using 20 ml of pH 6.8 phosphate buffer as a dissolution medium maintained at $37^0C \pm 1^0C$. The stirring speed was kept constant at 50 rpm. At predetermined intervals of study (1 to 5 min), 1 ml of sample was withdrawn and after every withdrawal 1ml of fresh dissolution medium was replaced. The samples were analysed either spectrophotometrically at 190-700 nm or was spotted for TLC analysis.

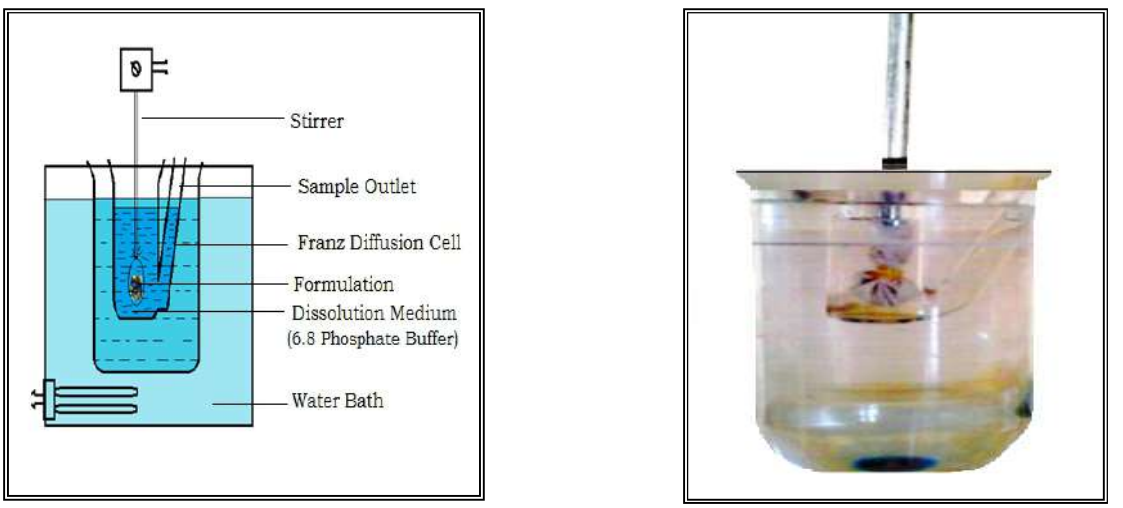


Figure 1: The modified apparatus for micro-dissolution study

Thin Layer Chromatography[30]

The extract was tested by thin-layer chromatography (TLC). Normal phase analytical TLC was performed on 3×5 cm silica gel (0.5 mm thickness) coated glass plates activated at 100-105 ^{0}C for 1 hr. Aq. Extract of shankhpushpi (as reference) was applied to the chromatographic plate (silica gel G, Merck) beside the dissolution sample taken at the end of 5 min. The plates were eluted in solvent system Toluene : Ethanol (7:3).The eluted plates were air dried and developed using vanillin: sulphuric acid (1 g :100ml) reagent followed by heating at 100°C for 10 minutes. The compounds were deleted by the characteristic colours of the spots and Rf values.

Sectrophotometric Evaluation (UV Analysis)

The extract was tested by Spectrophotometric Evaluation (UV Analysis). The dissolution sample at predetermined interval of study (1–5 min), after every withdrawal the samples were analysed spectrophotometrically at 190-700 nm.

In-vitro dissolution study of pellets of Shankhpushpi extract

Standard calibration curve of Shankhpushpi extract

Preparation of stock solution

About 100mg of shankhpushpi Extract was weighed accurately and was then dissolved in distilled water in a 100 ml volumetric flask having concentration 1000 µg/ml. From this 1 ml solution was pipetted out and diluted upto 10 ml to prepare stock solution having concentration 100 µg/ml.

Preparation of working standard solutions

About 1, 2, 3, 4 and 5 ml solution were taken from stock solution and diluted separately with distilled water in 10ml volumetric flasks to prepare the series of working standard solutions in concentration range of 10 to 50 µg/ml.

In-vitro Drug Release Study

The ability of the enteric coated pellets of Shankhpushpi extract (SP/I-02 to remain intact and to release the active ingredient in the physiological environment of intestine was assessed by conducting *in-vitro* drug release studies under conditions mimicking mouth to intestine. The drug release studies were carried out using USP standard dissolution apparatus-II (basket method) at stirring speed of 50 rpm at 37^{0}C ±1^{0}C in 900ml of dissolution medium. Initially the pH of dissolution was kept 1.2 for 2 hr using 0.1M Hydrochloric acid as the average gastric emptying time was estimated as 2 hrs. After two hours the pH of dissolution medium was adjusted to 6.8 using 1M NaOH solution and dissolution was continued. At predetermined intervals of study, 1 ml of sample was withdrawn at periodic intervals and it was made up to 10 ml with buffer solution. After every withdrawal 1ml of fresh dissolution medium was replaced. The samples were analysed spectrophotometrically at 248 nm. The dissolution experiments were conducted in triplicate and the means of the absorbance were calculated.

Anthelmintic Screening of Formulations

For screening of anthelmintic activity, 500 mg of pellets (Table 6.10) (containing Shankhpushpi extract) were crushed and to it 25 ml of distilled water was added. This mixture was added into a petridish containing 6 equal size earthworms of 3-5 cm in length and 0.1-0.2 cm in width to observe the paralysis and death time.

Table 4: Anthelmintic activity of bitter herb pellet formulations

Groups	Formulations	Actual Dose
1	Control	25 ml distilled water
2	Albendazole solution	16 mg/ml
3	SP/I-02	500mg pellets/25ml
4	SP/I-02	600mg pellets/25ml

The above doses were given to all the groups except Group 1.

RESULT AND DISCUSSION

Preliminary Phytochemical Evaluation

The extract obtained was subjected for preliminary phytochemical evaluation such as loss on drying (LOD), ash value and total bitters. The results obtained from these studies are tabulated in Table 5.

Table 5: Physical characteristics of Shankhpushpi extracts

Parameters	Shankhpushpi
Loss on Drying	3.23 %
Ash Values	8.63 %
Total Bitters	3.61 %

Standard procedures were adopted to carry the preliminary phytochemical investigation. The results of phytochemical studies obtained from these studies are tabulated in Table 6.

Table 6: Qualitative Chemical Tests for Extracts of Anthelmintic Drugs

Constituents	Aq. Shankhpushpi Extract
Flavonoids	+
Triterpenoids	_
Tannins	+
Amino Acid	+
Alkaloids	+
Carbohydrates	+
Glycosides	_
Saponins	+
Phytosterols	+
Phenols	+
Phlobatannins	_
Quinones	_
Oxalate	_

UV spectrophotometric study on the extract

A very dilute solution of shankhpushpi extract in water (about 50 µg/ml) shows absorption maxima at 248nm.

The Anthelmintic activity was carried out and compared with standard drug Albendazole. The result from the study is tabulated in Table 7 and graphically depicted in Figure 2.

Table 7: Anthelmintic activity of Shankhpushpi extracts

Sr. No.	Treatment	Concentration	Pheretima posthuma Groups (Earthworms)	
			Time taken for paralysis (min.sec)	Time taken for death (min.sec)
1	Control (D. Water)	---	---	---
2	Albendazole	**16mg/ml**	**189.33**	**371.50**
3	Aqueous Extract	0.02ml/ml	255.30	442.50
		0.04ml/ml	223.20	408.25
		0.08ml/ml	198.54	385.51
		0.1ml/ml	**182.35**	**372.12**
4	Marketed Albendazole tablet	**16mg/ml**	**191.5**	**397.25**

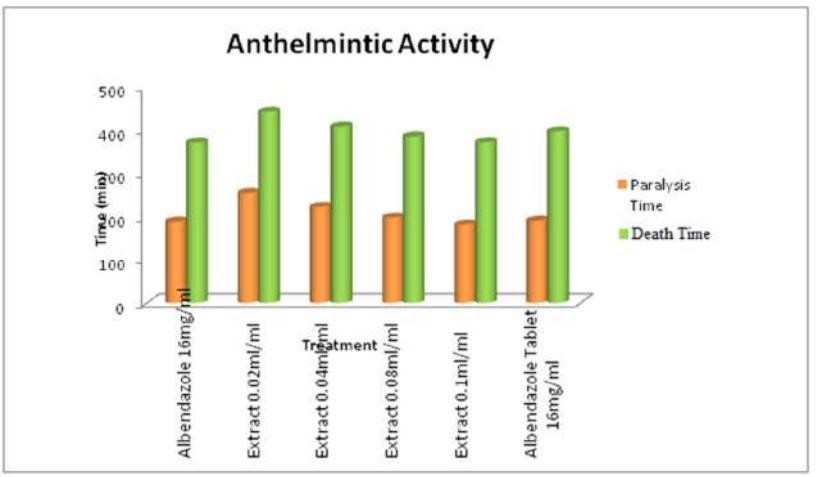


Fig. 2: Anthelmintic activity of Shankhpushpi extract.

On screening of prepared extract, the anthelmintic activity was observed on 0.1ml/ml as similar to the activity as that of Albendazole. The anthelmintic effect of Aqueous extract of shankhpushpi on the survival of earthworms (Table 7) was time and dose dependent. The higher doses of extract resulted in an early onset of activity and higher number of death of earthworms occurred compared with lower doses. The Aqueous extract of shankhpushpi at 10 mg/ml exhibited paralysis of earthworms on 182.35 min after exposure and death on 372.12 min of exposure. All the earthworms exposed to Albendazole solution were found to be paralyzed at 189.33 min and their death at 371.5 min, whereas, none of the earthworms was found dead or paralyzed in Distilled Water. And the solution of marketed tablet of Albendazole also exhibited significant activity.

The Aq. extract was selected for further experiment because its total bitter value was more and optimum anthelmintic activity.

Pellet Formulations of Bitter Herb

All pellet formulations were prepared using Aq. extract. Formulations were coded as SP/I -01 to SP/I -03 and SP/I - 02a to SP/I - 02d for Taste Masking method.

Characterisation of Pellets

The evaluation results of pellet formulations are as follows:

Table 8: Evaluation results of taste masked pellet formulations

Formulation Code	Bulk Density	Tapped Density	Hausner Ratio	Carrs Index
SP/I -01	0.701 ± 0.105	0.784 ± 0.087	1.1184	10.5867
SP/I -02	0.645 ± 0.069	0.727 ± 0.077	1.1271	11.2792
SP/I -03	0.689 ± 0.076	0.769 ± 0.113	1.1161	10.4031
SP/I -02 a	0.634 ± 0.089	0.701 ± 0.045	1.1056	9.5577
SP/I -02 b	0.677 ± 0.112	0.740 ± 0.086	1.0930	8.5135
SP/I -02 c	0.645 ± 0.069	0.727 ± 0.077	1.1271	11.2792
SP/I -02 d	0.689 ± 0.098	0.754 ± 0.057	1.0943	8.6206

*All values are average of triplicates.

Table 9: Evaluation results of taste masked pellet formulations

Formulation Code	Angle of Repose	Flow Rate (g/sec)	Friability (%)	Avg. Diameter (µm)
SP/I -01	27.87 ± 0.599	2.857 ± 0.026	0.72	712.56
SP/I -02	26.98 ± 0.625	3.334 ± 0.017	0.64	740.32
SP/I -03	27.36 ± 0.637	2.500 ± 0.021	0.69	705.36
SP/I -02 a	27.19 ± 0.913	2.851 ± 0.037	0.76	751.54
SP/I -02 b	27.45 ± 0.847	2.223 ± 0.043	0.81	709.89
SP/I -02 c	26.98 ± 0.625	3.334 ± 0.017	0.64	740.32
SP/I -02 d	28.02 ± 0.713	1.8182 ± 0.029	0.86	698.78

*All values are average of triplicates.

Evaluation of taste masking of Shankhpushpi pellets

Gustatory Sensation Test for Taste Masking

The taste perception studies in order to evaluate the efficiency taste masked formulations of pellet of shankhpushpi extract was carried out using Hedonic scale rating. By selecting the appropriate excipients, it was possible to modify the apparent taste of pellet formulations of extract.
In the taste masking pellet composition SP/I-02 was efficiently taste masked based on values as shown in Table 10.

Table 10: Evaluation of Taste Masking Performance of shankhpushpi pellets

Formulation Code	SP/ I -01	SP/ I -02	SP/ I -03
Rating	5	6	4

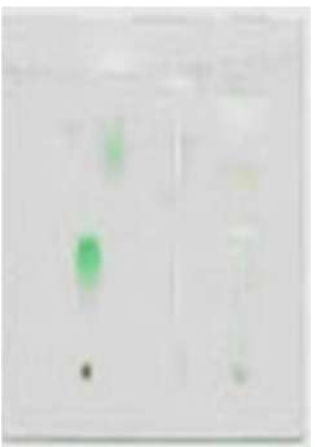
Table 11: Evaluation of Taste Masking Performance of optimized Shankhpushpi pellets

Formulation Code	SP/ I -02 a	SP/ I -02 b	SP/ I -02 c	SP/ I -02 d
Rating	6	6	6	5

Chromatographic evaluation of formulations by TLC

Chromatographic evaluation of Extract and formulations was done using Thin Layer Chromatography (TLC). The compounds were detected by the characteristic colours of the spots and Rf values.

Mobile Phase- Toluene : Ethanol (7:3)



Ref. with Sample at 5 min.

Figure 3: TLC of Shankhpushpi Extract and 5 min dissolution sample of pellets in Toluene : Ethanol (7:3)

Table 12: Rf values of Standard Shankhpushpi extract

	TLC	Sample
Rf Values of Shankhpushpi	Reference	
	0.81 (Green)	No spot appear
	0.40 (Green)	

When TLC study were conducted by selecting Toluene : Ethanol (7:3) as mobile phase. Results obtained are depicted in Fig. 3 and Rf values of reference were tabulated in Table 12. It shows maximum Rf values of various colours. When the sample of 5 min micro-dissolusion was run with reference of same concentration, the sample run of 5 min micro-dissolution of taste mask pellet formulation does not show the spots and reference shown the 2 spots having different Rf values.
The chromatographic evaluation shows that the Pellet formulation was taste masked since no bitter was released within 5 min. period.

Spectrophotometric Evaluation (UV Analysis)

The pellets were subjected to micro-dissolution test and samples in time interval 1 to 5 min were analysed spectrophotometrically for release of bitters from the taste mask formulation. The absorption spectra of taste masking pellet formulation does not show any absorbance it may be concluded that the bitter taste would be masked in-vivo.

In–vitro dissolution study of Shankhpushpi Pellets

The in–vitro dissolution study of Shankhpushpi was carried out using SP/I – 02, Pellets formulation.

Standard calibration curve of Shankhpushpi Extract

In distilled water shankhpushpi extract showed the absorption peak at 248 nm. The R^2 values for shankhpushpi extract calibration curves were found out to be 0.9819 in distilled water

In-vitro drug release study

Table 13: % Release of Shankhpushpi Extract at 248 nm

Sr. No.	Time Point (min)	% released of Shankhpushpi Extract (248 nm)		
		Formulation code		
		SP/I- 01	SP/I- 02	SP/I- 03
1	0	0.00	0.00	0.00
2	5	0.70	0.00	0.52
3	60	1.08	0.00	0.96
4	120	1.45	0.00	1.24
5	135	32.01	17.86	9.27
6	150	33.56	29.38	19.43
7	165	45.30	32.26	25.23
8	180	53.91	35.14	30.96
9	195	57.82	46.67	38.61
10	210	59.38	52.43	44.35
11	225	61.58	55.31	46.26
12	240	67.21	72.59	52.56
13	255	72.65	90.68	58.24

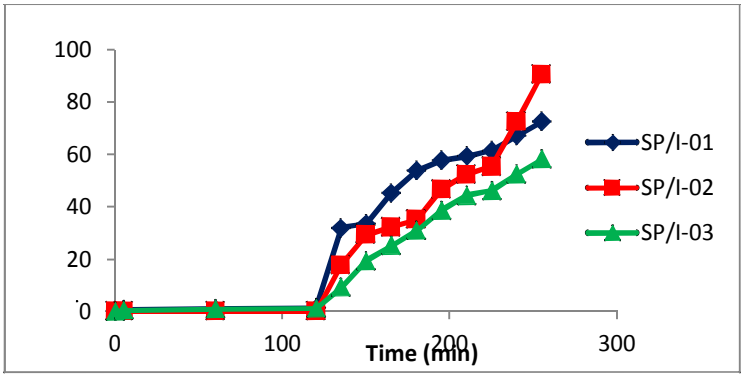


Figure 4: % Release of Shankhpushpi Extract at 248 nm.

The formulated taste mask pellets of Shankhpushpi was subjected to in-vitro drug release studies to study the release of the active constituents of shankhpushpi extracts in the physiological environment of mouth, stomach and intestine i.e. at pH 6.8, pH 1.2 and pH 6.8 phosphate buffer. The percentage of shankhpushpi extract released for first 2 hours from the above formulations in pH 6.8 and pH 1.2 was negligible. In the pH 6.8 drug started to release from all the batches of pellet formulations. The total amount of drug release from SP/I-01, SP/I-02 and SP/I-03 was found to be 72.65, 90.68 and 58.24 % after 255 min at 248 nm respectively. The maximum percentage of drug release was observed from formulation SP/I – 02. The results of the dissolution studies indicates that the optimized pellet formulation SP/I – 02 has better dissolution profile as compared to SP/I-01 and Sp/I-03.

Anthelmintic Screening of Pellet Formulations

Anthelmintic activity was carried out using Earthworms and compared with standard Albendazole solution, results of the study parameters are summarized in Table 14 and Figure 5.

Table 14: Anthelmintic activity of bitter herb pellet formulations

Sr. No.	Group	Concentration mg/ml	Pheretima posthuma Groups (Earthworms)	
			Time taken for paralysis (min)	Time taken for death (min)
1	Control (D. Water)	---	---	---
2	**Albendazole**	**16mg/ml**	**189.33**	**371.50**
3	SP/I–01	500mg pellets/25ml	202.45	399.00
		600mg pellets/25ml	195.23	368.25
4	SP/I–02	**500mg pellets/25ml**	**191.20**	**375.5**
		600mg pellets/25ml	183.52	364.2
5	SP/I–03	500mg pellets/25ml	235.58	412.36
		600mg pellets/25ml	209.00	398.30

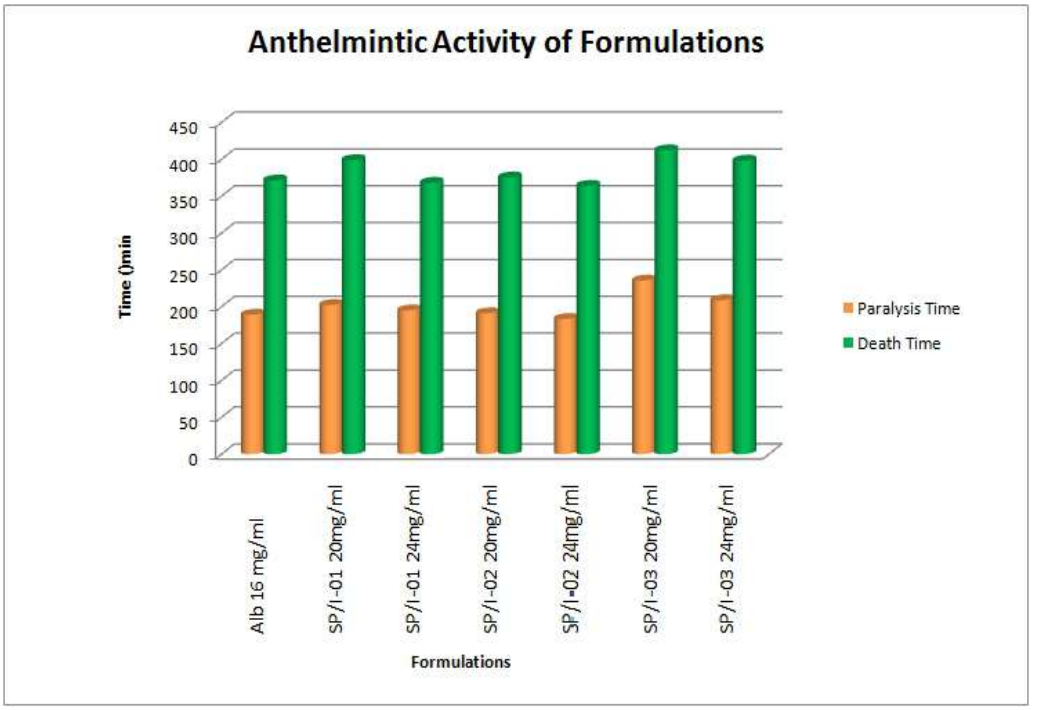


Figure 5: Anthelmintic activity of bitter herb pellet formulations

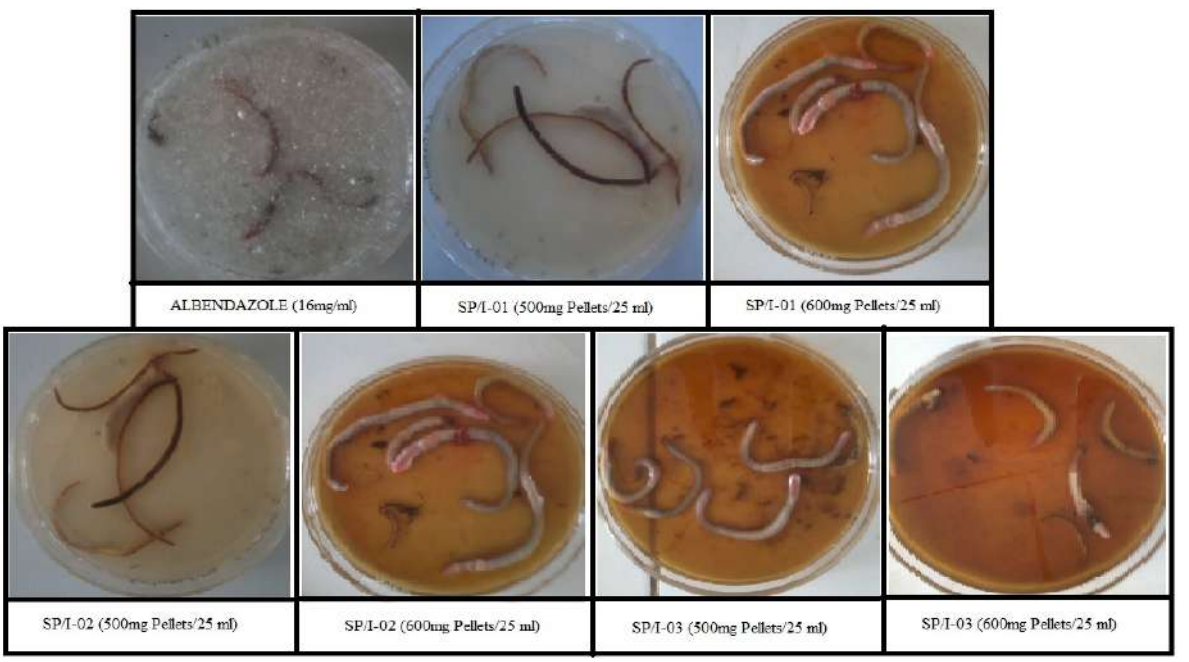


Figure 6: Anthelmintic activity of bitter herb pellet formulations

Anthelmintic activity of formulation and comparison with standard (Albendazole) indicated that, optimized formulation exhibited comparable and significant activity. From the study it can be concluded that, the taste masking formulation SP/I-02 showed highest activity.

CONCLUSION

Helminthic infections are among the most common infection in human being, especially in children. But the lack of anthelmintic pediatric formulations is a big concern all over the world. Most of the existing dosage forms are not flexible for dose adaptation to the child bodyweight. Tablet breaking is a frequent method to obtain the desired dose that breaking of tablets (proposed as means for dose adaptation to children) resulted in a weight variation which may compromise the clinical outcome.

All over the world there is increasing tendency towards consuming natural products and thus living a natural life. Ancient classical literature and ethnomedical surveys described the use of plants in traditional system of medicines for the treatment of helminthic infections. But most of the herbal anthelmintic drugs possess bitter

taste. Thus, another challenge in the development of pediatric anthelmintic formulations is the unpleasant taste that can lead to the poor compliance. In the present study, efforts was made to standardize the plant extract with good anthelmintic activity and formulated best alternative herbal preparations to replace or complement the synthetic drugs which are currently in use. Shankhpushpi pellets were proposed as alternatives to tablets breaking, as they offer more flexibility for dose adaptation to a child's body weight. They were produced by taste masking method via extrusion-spheronization. Based on dissolution and the chromatographic data, the taste masking efficiency of pellets was evaluated

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