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# Pharmacognostic and Phytopharmaceutical Studies of *Alocasia Fornicata* and Its Anti-Diarrheal Effect on Castor Oil Induced Diarrhea in Rats

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***Abstract:***
***Objective:*** *Present study focused on development of herbal capsules using Alocasia fornicate pure compounds and evaluate the Anti-diarrheal effect of Alocasia fornicata formulation using castor oil induced diarrhea in rats.*
***Material and Method:*** *Isolate the pure compounds from Alocasia fornicata whole plant using column chromatography. Pure compounds were used for herbal capsule formulation and performed quality control tests. Developed formulation was used to evaluate Anti-diarrheal effect castor oil induced diarrhea in rats.*
***Results:*** *β–Sitosterol derivative were isolated from Alocasia fornicata whole plant and confirmed by analytical data. Developed herbal capsule formulation was found in an acceptable range of drug content, uniformity of weight and disintegration time. DSC study revealed stability of the drug in the presence of excipients. Alocasia fornicata capsules showed significantly decreases peristaltic activity of small intestine as a result of permeability of Na+ and Cl- changed in the intestinal mucosa against ricinoleate induced diarrhea.*
***Conclusion:*** *Developed Alocasia fornicata capsules exhibited unique Anti-diarrheal effect using castor oil induced diarrhea in rats.*
***Keywords:*** *Alocasia fornicate, Castor oil, Diarrhea, Peristaltic activity, Drug content etc.*

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## I. Introduction:

Herbal medicine or Phytomedicine is the use of plants for medicinal and therapeutic purpose for curing of diseases and improve human health[1]. Plants have secondary metabolites called phytochemicals. *Alocasia fornicata* is a plant species of many-nerved, broad-leaved, rhizomatous or tuberous perennials from the family Araceae, native to Indochina and to the Indian Subcontinent[2]. It characteristically grows 2' - 3 ' in height with slightly pink petiole, triangular wide shaped leaves and a horizontally growing stolon. The plant contains flavonoids, cynogenetic glycosides, ascorbic acid, gallic acid, mallic acid, oxalic acid, alocasin, amino acids, succinic acid, and β-lectines. Young leaves contain up to 0.018% hydrogen cyanide and a mixture of triglochinin and iso-triglochinin. *A. fornicata* possesses antibacterial[3] and cytotoxic activities[4], A. micorrhiza possess antioxidant, antimicrobial, thrombolytic, cytotoxic and anthelmintic activity, Antihyperglycemic activity, Analgesic activity, Antioxidant[5] and antibacterial activity. Major compounds identified in the ethanol extract of *A. indica* corm included: β-hydroxyque brachamine[6], gibb-3ene-1,10- dicarboxylic acid,2,4 α-dihydroxy-1-methyl-8- methylene,14α- lactone,10methyl ester (3.8 %), 4,4-dithiobis butanoic acid (2.6 %), 5-( *p* -aminophenyl)-4-( *O* -tolyl)- 2 thiazolamine (8.4 %), 3H-1,4-benzodiazepine-2- amine,7-chloro- N -methyl-5-phenyl,4-oxide (4.7 %), 10, 12, 14 nonacosatriynoic acid (15.3 %), androst- 4- en-11-ol-3,17-dione,9-thiocyanato (16 %), estra- 1,3,5(10)-trien-17-one,3-hydroxy-6-methoxy, *O* - methyloxime (29.5 %), 17 α-ethynyl-17 β- hydroxy-6-β-methoxy-3 α, 5 cyclo-5 α-androstan- 19-oic acid (tr), 1H indole,3 benzyl -2-phenyl (tr) and 1H indole, 5 methyl-2,3- diphenyl (tr) .

The plant contains flavonoids, cynogenetic glycosides, ascorbic acid, gallic acid, mallic acid, oxalic acid, alocasin, amino acids, succinic acid, and β-lectines, bioactive vegetable oil[7,8]. Young leaves contain up to 0.018% hydrogen cyanide and a mixture of triglochinin and iso-triglochinin. All parts of the plant, except rhizome, contain cyanogenic principle. Research study shows that an anti-fungal protein designated alocasin

was isolated from the rhizomes of the giant taro *Alocasia macrorrhi*. A lectin from the tubers of *Alocasia indica* Schott has also been purified by affinity chromatography on asialofetuin-linked amino activated silica beads.

As the drug is endowed with colossal exploitation and utilization value, it is medicinally vital to know precisely and comprehensively about its characteristics of pharmacognosy. Here in we made a detailed investigation on pharmacognostic, phytochemical, physicochemical, fluorescence analysis and thin layer chromatography of this plant to help in its identification and standardization and research was performed to be able to analyze the therapeutic potential of the plant with regards to its formulation, evaluation and pharmacological activity[9,10].

## II. Materials And Methods

*Collection and identification of plant material*

Fresh plants of *Alocasia fornicata* roxb was collected from Tirumala, Tirupati, Andhra Pradesh, identified and authenticated by Prof. K. Madhava Chetty, Plant Taxonomist (IAAT:1324), Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

*Preparation of various extracts*

The whole plant of *A. fornicata* was collected, cleaned; dried in the shade at room temperature and chopped into small pieces. Dried pieces of roots and whole plant were powdered and then filled in the airtight bottle. The coarse powders of both plant materials subjected to maceration for 24 hr sequentially with solvents based on polarity, i.e., petroleum ether, chloroform, ethyl acetate, n-butanol, methanol, and water. Thereafter, the extracts were concentrated by using rotary vacuum evaporator (50 $^{o}$C).

*Preparation of sample*

*Transverse section*

The Transverse sections were taken by placing the *A. fornicata* root/stem between the thumb and four fingers of the left hand (for *A. fornicata* leaf use pieces of potato and put the leaf in an incision made on potato piece). Applying sharpened blade kept in the right hands, thinner sections were performed finally, the blade over the object in quick successions. The sections had been transmitted into watch glass comprising normal water, applied chloral hydrate to these sections, boiled, strained as well as the sections had been marked with the phloroglucinol and hydrochloric acid (1: 1) and the same is mounted in glycerin and then viewed using low power. In order to supplement the illustrative component, photomicrographs in various magnification of most important tissues and cells had been taken. For the normal histological purpose, sections had been took pictures of under the bright field light.

*Powder microscopy*

The shade dried leaves, root and stem of *A. fornicata* had been powdered and the powders that pass through sieve number 60# individually and then afflicted by powder analysis. All the powders had been taken up with few drops of chloral hydrate had been added and warmed for one to two minutes. Chloral hydrate utilized to clear the tissues and for clarification. To the cleared powder, one or two drops of 1: 1 combination of phloroglucinol and HCl had been added after which it had been lastly fastened along with glycerin. Lignified tissue obtained pink color. As a way to study the starch grains, the powder had been fastened together with water and 1-2 drops of dilute iodine, although to see the calcium oxalate crystals unstained sections had been fastened merely together with water.

*Quantitative microscopy*

Quantitative microscopical analyses of *A. fornicata* leaves had been carried out as well as following variables have been determined.

*Stomatal number*

It is the average number of stomata per square millimeter of the epidermis of the leaf. Part of the leaf (middle part) had been cleared through boiling along with chloral hydrate solution or otherwise with chlorinated soda.

*Stomatal index*

Stomatal index is the percentage, which the number of stomata forms to the total number of epidermal cells, every stomata getting mentioned as one cell.

*Vein-islet number*

A vein-islet is the small part of green tissue between the veinlets. The vein-islet number is the average number of vein-islets per square millimeter of a leaf surface.

*Veinlet termination number*

Veinlet termination number is described as the number of veinlet termination per mm$^2$ of the leaf surface, midway between midrib of leaf and its margin.

*Proximate analysis*

Proximate evaluation helps to established specific standard regarding dried crude drugs to prevent batch-to-batch variance and to assess their quality. Their analyses additionally provide a concept concerning the nature of phytoconstituents found. Proximate analysis of *A. fornicata* leaf, stem and root powders had been performed utilizing strategies recommended in the WHO quality control of herbal materials by subjecting these to several determinations such as

a. Total Ash
b. Acid-insoluble ash
c. Water soluble ash
d. Alcohol soluble extractive value
e. Water-soluble extractive value
f. Loss of moisture content

*Phytochemical studies*

*Successive solvent extraction*

5 Kg the air-dried powdered of *A. fornicata* had been sequentially extracted using the subsequent solvents of increasing polarity in a cold maceration a) Petroleum ether b) Chloroform d) Ethyl acetate e) n-butanol f) Methanol g) Water. All of the extracts had been concentrated by distilling the solvents and the extracts had been dried with the help of rota-evaporator. Every time prior to extracting using the subsequent solvent, the marc had been dried in an air. The marc had been eventually macerated together with water for 24h to get the aqueous extract. The completing the extraction had been affirmed by evaporating one or two drops of extract from the thimble on watch glass to see that no residue remained soon after evaporation of the solvent. The liquid extracts acquired with various solvents had been obtained.

*Preliminary Phyto-chemical screening*

The extracts were then subjected to various qualitative tests using as per following to determine the presence of various phytoconstituents such as alkaloids, glycosides, flavonoids, carbohydrates, amino acids, saponins, sterols and terpenoids, cardiac glycosides, coumarins, carotenoids, tannins, phenolic compounds, fixed oils and fats etc.

*Fluorescence analysis*

Fluorescence evaluation was carried out by ultrasonic processing the powder drug with different reagents and then detected at 254 nm, 366 nm within an ultraviolet chamber and visible light (20-22).

*Isolation of compounds*

5 gm of Methanolic extract *A. fornicata* was subjected to silica-gel (100–200 mesh) column (length 100 cm and diameter 3 cm) chromatography and The elution started with hexane followed by hexane- Ethyl acetate (EtOAc) mixtures (9 : 1, 8: 2; 7: 3, 6: 4, 5: 5, 4: 6; 3: 7, 2: 8, 1: 9), EtOAc (100%), EtOAc-methanol (MeOH) mixtures (9: 1, 8: 2 and 7: 3), and ended with MeOH (100%). A total of 55 fractions, 100 mL each, was collected from the tubes; those with similar thin layer chromatography (TLC) profiles were combined fractions as F1 l-6 (Pl); F2 7-12 (P2); F3 13-18 (P3), F4 19-32 (P4), F5 33-45 (P5); F34-43. From the five pooled fractions (Pl to P5) eluted with hexane-EtOAc mixtures (9:1) eluted two compounds, further these fraction subjected to column chromatography with hexane: chloroform mixure (9:1, 5:5, 1:9). In these fraction, white crystal powder (164 mg) was eluted from hexane: chloroform (9:1) with melting point 65$^{o}$C. White solid (60 mg) was eluted from hexane: EtOAc mixtures (6:4) with melting point 135 °C. These compounds were subjected to Mass spectroscopy, FTIR, $^{1}$H NMR for structure analysis.

**Development of formulation of isolated compound[11]**

**Preparation of β-sitosterol granules**

β-sitosterol granules were prepared by wet granulation method.

Granules were also prepared containing sodium starch glycollate (SSG) as super disintegrant. SSG is incorporated at different concentration (2%, 3%, 5%) separately and granulations were carried out similar way as described above.

**Evaluation of β-sitosterol granules**

Prepared β-sitosterol granules were subjected for determination of bulk density, tapped density, Hausner ratio, Carr's index, angle of repose in order to assess the flow property of granules.

**Formulation of β-sitosterol capsules[12]**

Prepared granules were packed into hard gelatin capsule (size 1) using hand operated capsule filling machine such that each capsule contains 400 mg of granules. β-sitosterol capsules without SSG were labelled as F1 and capsules containing 2%, 3% and 5% of sodium starch glycolate (SSG) were labelled as F2, F3, and F4 respectively. Estimation of drug content (β-sitosterol) in capsules[13] Granules from 10 capsules were mixed and

weight of powder equivalent to 5 mg of β-sitosterol and extracted with the phosphate buffer of pH 6.8 for 30 min. These solutions were filtered, suitably diluted and absor-bance was measured at 208 nm against blank solution (phosphate buffer pH 6.8) using a UV spectrophotometer.

**Determination of uniformity of weight**

Twenty capsules were selected. Each capsule was weighed on an analytical balance, carefully emptied of its content, the shells reweighed and the weight of content determined. The collective weight of content, average weight of content per capsule and the deviations (%) of individual content weights from the mean were calculated.

**Determination of disintegration time**

Disintegration times for capsules were determined by disintegration apparatus. Six capsules were placed in six tubes of the basket and the apparatus was operated using water as release medium maintained at 37 ± 2°C. The capsules were observed and the times taken for complete disintegration of all capsules were determined.

***In vitro* dissolution study**

*In vitro* dissolution study of all the prepared capsule formulations was done using USP Type II paddle dissolution apparatus (Electrolab USP dissolution tester TDT-08L) using 900 ml phosphate buffer pH 6.8 at 100 rpm and results were compared with drug release of β-sitosterol from capsule formulation F0. An aliquot amount of the sample was withdrawn at regular time intervals and the same volume of pre-warmed (37±0.5ºC) fresh dissolution medium was replaced. The samples were filtered, suitably diluted and β-sitosterolin each sample was analyzed by using Shimadzu UV-spectrophotometer at 208 nm.

**Anti diarrheal activity of developed formulation**

**Castor oil-induced diarrhea in rats**[14, 15, 16]

Castor oil-induced diarrhea was done according to the method of (Shoba FG, Thomas M 2001). Rats of either sex were divided into four groups of five rats each.

The animals were fasted for 18 h prior to the test.

Group I was treated with normal saline (2 mL/kg), which served as control

Group II received loperamide (5 mg/kg)

Groups III and IV received Formulation 4 (5 mg/ kg and 50 mg/kg). All doses were administered orally. After 1 h, all groups received 1 mL of castor oil orally. Then animals were placed in cages lined with adsorbent papers and observed for 4 h for the presence of diarrhea defined as watery (wet), unformed stool. The control group result was considered as 100%. The activity of each group was expressed as percent inhibition (%) of diarrhea. The percent inhibition of defecation was calculated as follows: % Inhibition of defecation=[(A-B)/A]X100 Where A indicated mean number of defecation caused by castor oil; B indicated mean number of defecation caused by drug or formulation.

**Castor oil-induced enteropooling**[17, 18]

The castor oil-induced enter pooling was carried out according to the method of Robert et al. Rats of either sex were divided into four groups and fasted for 18 h prior to the test. Group I as control (saline 2 mL/kg body weight, orally); Group II received standard drug (Loperamide 5 mg/kg body weight, orally), (Groups III and IV) were given Formulation 4 (5 mg/ kg and 50 mg/kg body weight, orally). One hour later, all the rats were challenged with 1 mL of castor oil orally. After 1 h of castor oil received, the rats were sacrificed and the small intestine from the pylorus to the caecum was isolated. Then the intestinal contents were weighed and volume measured by graduated tube.

**Gastrointestinal motility test**[19]

This test was performed according to the method previously described using charcoal as a diet marker. Animals of either sex were divided into four groups of five rats in each and fasted for 18 h before test. All groups received castor oil to produce diarrhea. One hour later,

Group I treated as control (saline 2 mL/kg body weight, orally);

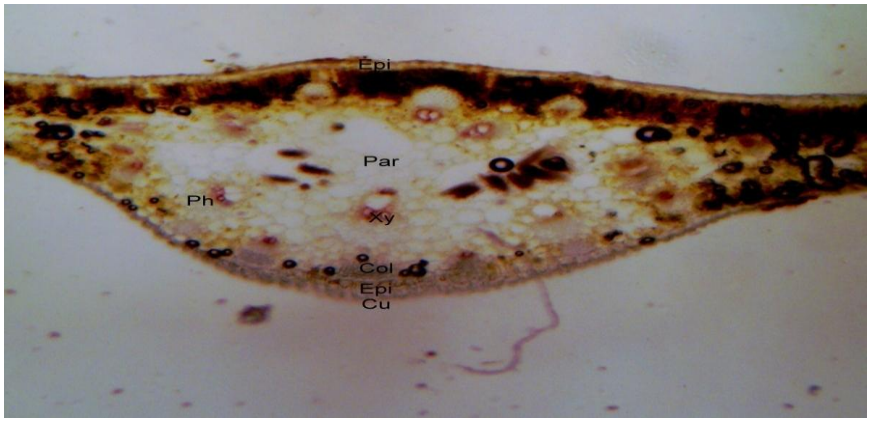
Group II received standard drug (Loperamide 5 mg/ kg body weight, orally) and the rest two groups received Formulation 4 (5 mg/ kg and 50 mg/kg).

After 1 h of drug administration, all animals were received 1 mL of charcoal meal (10% charcoal suspension in 5% gum acacia) orally. One hour later, all animals were sacrificed, and the distance covered by the charcoal meal in the intestine from the pylorus to the caecum was measured and expressed as Percentage of distance moved.

**Statistical analysis**

Graph Pad prism 5.0 (Graph Pad Software Inc., La Jolla, CA, USA) was used to analyze the data obtained in this study. One-way analysis of variance (ANOVA) was employed, followed by TUKEY test. Data are presented as mean ±standard deviation (SEM) and values of $p<0.05$ were considered significant.

## III. Results




**Leaf**

**Fig no 1:** Detailed TS of midrib of leaf showed upper epidermis, collenchyma and parenchymatous cells. Cu: Cuticle; Epi: epidermis; Col: Collenchyma; Par:

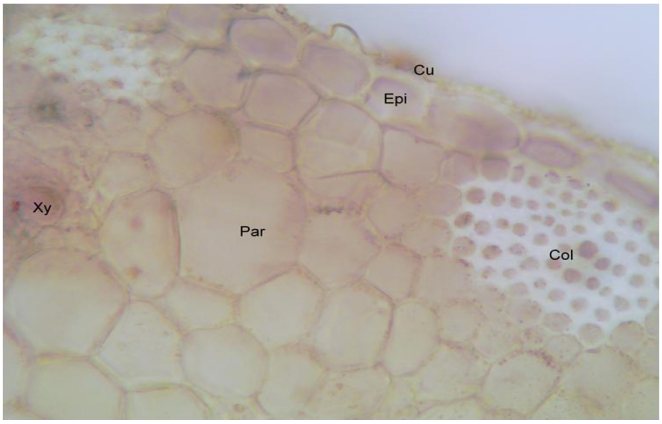



**Fig no 2:** Parenchyma. Transverse Section Midrib portion of A. fornicata leaf. Cu: Cuticle; Epi: Epidermis; Par: Parenchyma; Xy: Xylem, Ph: Phloem; Col: Collenchyma.

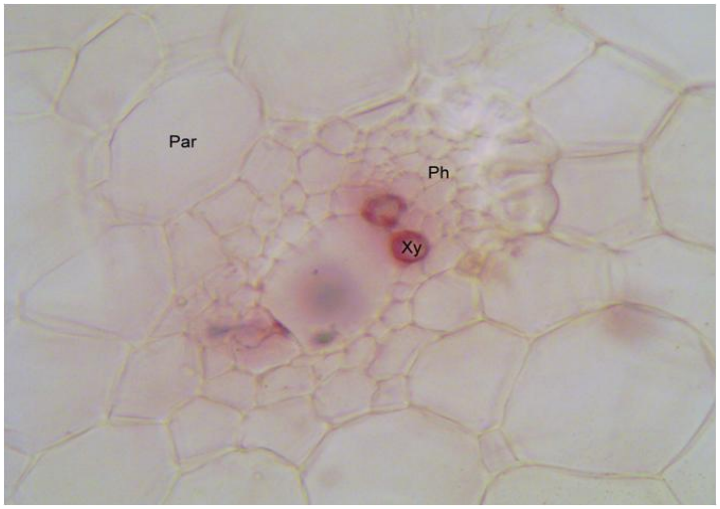



**Fig no 3:** T.S of Midrib portion of A. fornicata showed Vascular Bundles Ph: Phloem; Xy: Xylem.

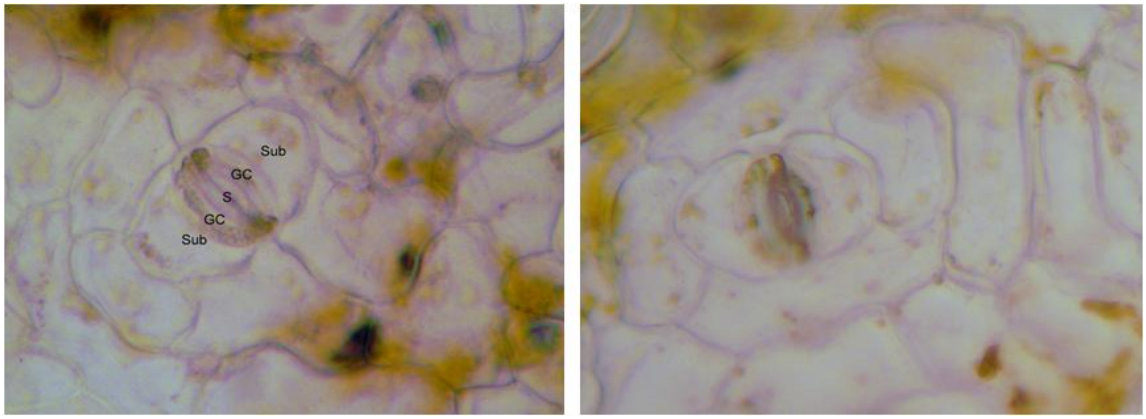



**Fig no 4:** Epidermis of leaf Paracytic Stomata

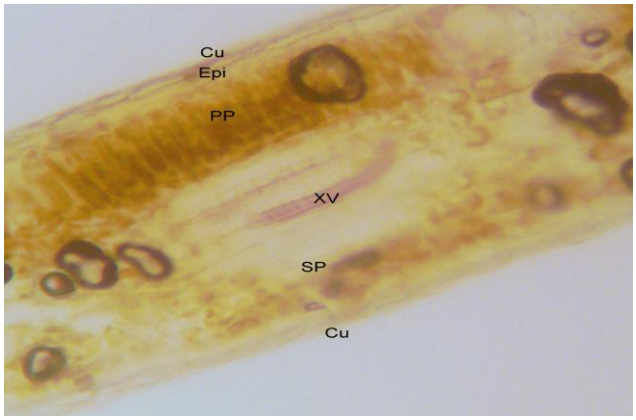



**Fig no 5: T**. S of Lamina of A. farnicata

TS of leaf passing through midrib region shows slight upper notch and large notch at lower surface. Upper and lower surface of the leaf consists of rectangular thin walled epidermis, covered with thick cuticle followed by collenchymatous ground tissue; palisade cells reached up to the upper notched region. Palisade cell is single layered. Vascular bundles are scattered in the ground tissue. Xylem is surrounded by phloem facing toward the lower side. Xylem consists of vessels, tracheids, fibers and xylem parenchyma; cells are parenchymatous. TS passing through lamina region showed single layered palisade cells followed by several layers of spongy mesophyll embedded with lateral vascular bundles. *A. fornicata* leaf surface shows the paracytic stomata which is characteristic of Family Araceae.

**Powder Microscopy of Leaf:** It revealed the presence of paracttic stomata, epidermal cells and lignified xylem vessels.

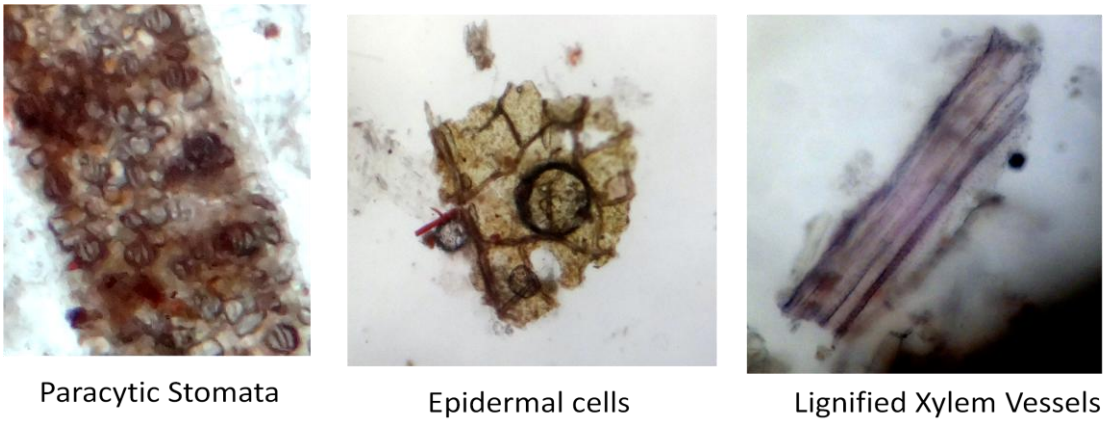



**Fig no 6:** Powder Microscopy of Leaf

**Root**

**Fig no 7:** Morphology of Root

Section of root is shown in above figure. The outer layer is composed of multilayered cork, followed by 7-8 layered collenchyma cells. Cells are oval in shape. Central large portion of stem are occupied by pith, outside of it hypodermis is present. Barrel shaped cells of pith store large amount of food. Vascular bundles are present at the center.

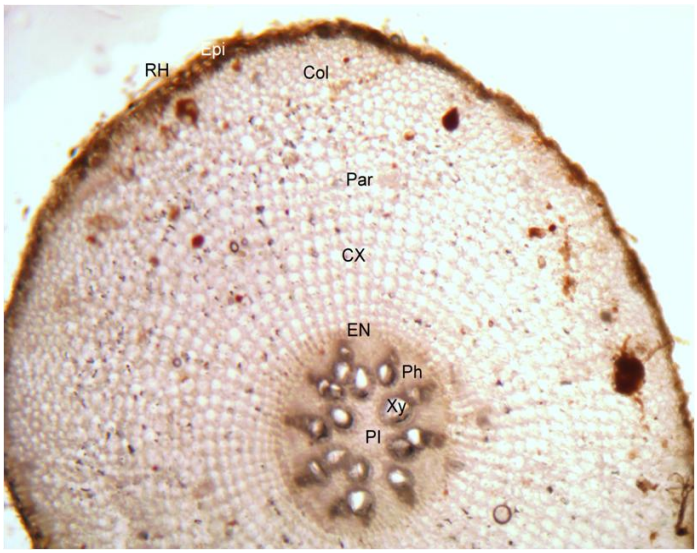



**Fig no 8:** T. S of Root

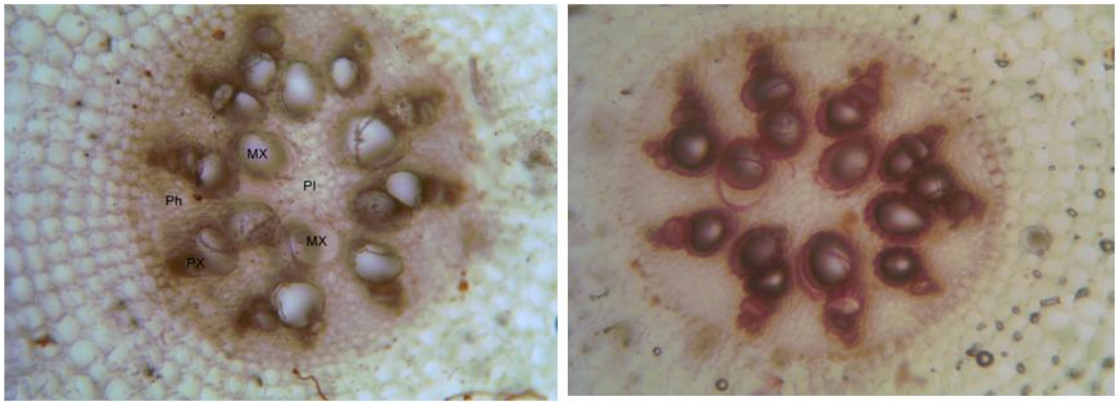



**Fig no 9:** Vascular Bundle region of Root

**Powder Miscroscopy of root**

Powder microscopy of root of A. fornicata revealed the presence of pigment cells, parenchyma, phloem fibers and calcium oxalate crystals.

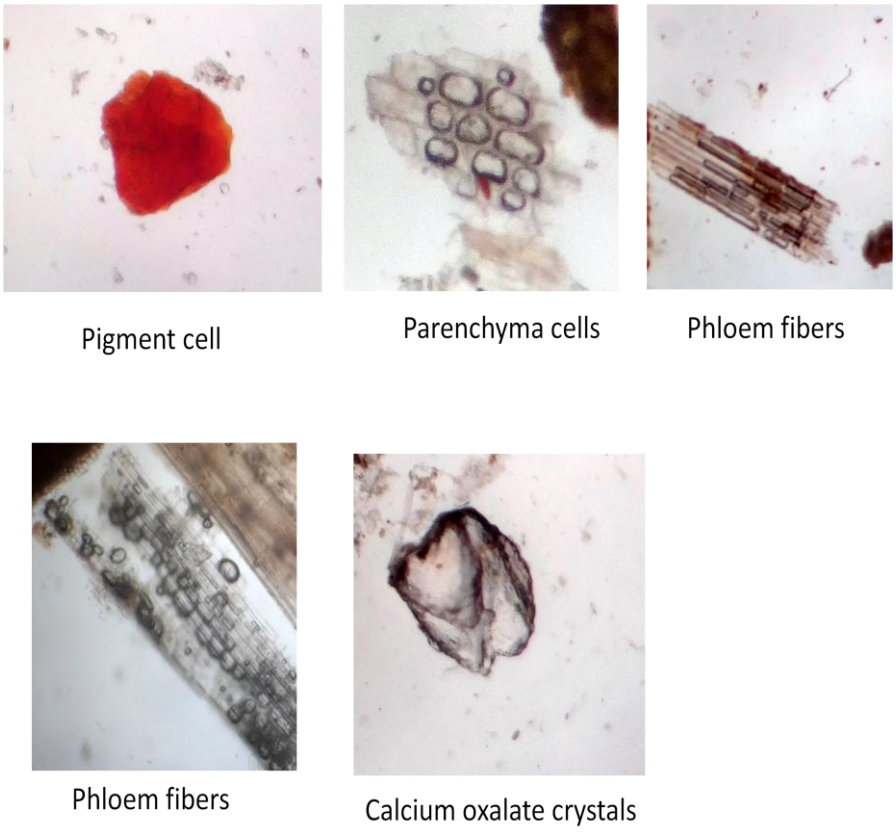



**Fig no 10:** Powder microscopy of root of *A. farnicata*

**Table no 1:** Preliminary Phyto-chemical screening

| Phytoconstituents | Method | Pet. ether extract | Ethyl acetate extract | Chloroform extract | Methanol extract | Aqueous extract |
|---|---|---|---|---|---|---|
| Flavonoids | Shinoda Test | - | + | - | + | + |
| | Zn. Hydrochloride test | - | + | - | + | + |
| | Lead acetate Test | - | + | - | + | + |
| Volatile oil | Stain test | - | - | - | - | - |
| Alkaloids | Wagner Test | - | - | + | + | + |
| | Hager's Test | - | - | + | + | + |
| Tannins & phenols | $FeCl_3$ Test | - | + | - | + | + |
| | Potassium dichromate test | - | - | - | + | + |
| Saponins | Foaming Test | - | - | - | + | + |
| Steroids | Salkowski test | + | - | + | + | + |
| Fixed oils and fats | Spot test | + | - | - | + | - |
| Carbohydrates | Molish test | - | - | - | + | + |
| Acid compounds | Litmus test | - | - | - | + | + |
| Glycoside | Keller-Killani Test | - | - | - | + | + |
| Amino acids | Ninhydrin test | - | - | - | + | + |
| Proteins | Biuret | - | - | - | + | + |

**+ Present and – Absent**

**Table no 2:** Proximate analysis

| Parameters | Values (%w/w) |
|---|---|
| Moisture content (Loss on drying) | 7.52±2.34 |
| Total ash | 6.85±1.52 |
| Acid insoluble ash | 3.66±0.72 |
| Water soluble ash | 2.12±0.55 |
| Petroleum ether soluble extractive value | 0.86±0.05 |
| Chloroform soluble extractive value | 2.06±0.06 |
| Ethyl acetate soluble extractive value | 3.85±0.82 |
| Alcohol soluble extractive value | 7.12±1.22 |
| Water soluble extractive value | 10.24±2.51 |

**Table no 3:** Fluorescence analysis of Whole plant of *Alocasia fornicata*

| Solvent used | Visible light | UV light | |
|---|---|---|---|
| | | 254nm | 366nm |
| Water | Buff | Brown | Black |
| NaOH | Dark Brown | Brown | Black |
| HCl | Reddish brown | Black | Orange –red |
| $HNO_3$ | Brown | Green | Dark green |
| $Fecl_3$ | Dark green | Light green | Black |
| Picric acid | Yellowish brown | Yellow | Dark brown |

**Table no 4:** Characterization of Isolated Compound

Physical properties of Isolated Compound

| S. No | Parameter | Observation |
|---|---|---|
| 1 | Colour | White |
| 2 | Shape | Crystalline |

**Table no 5:** Chemical Tests of Isolated Compound

| S. No | Test | Results |
|---|---|---|
| 1 | Salkowaski | + |

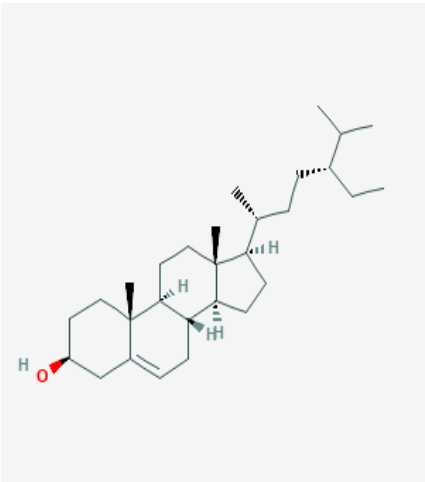



**Fig no 11: Chemical Structure of β-Sitosterol derivative**

**(IUPAC Name:** 17-(5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol)

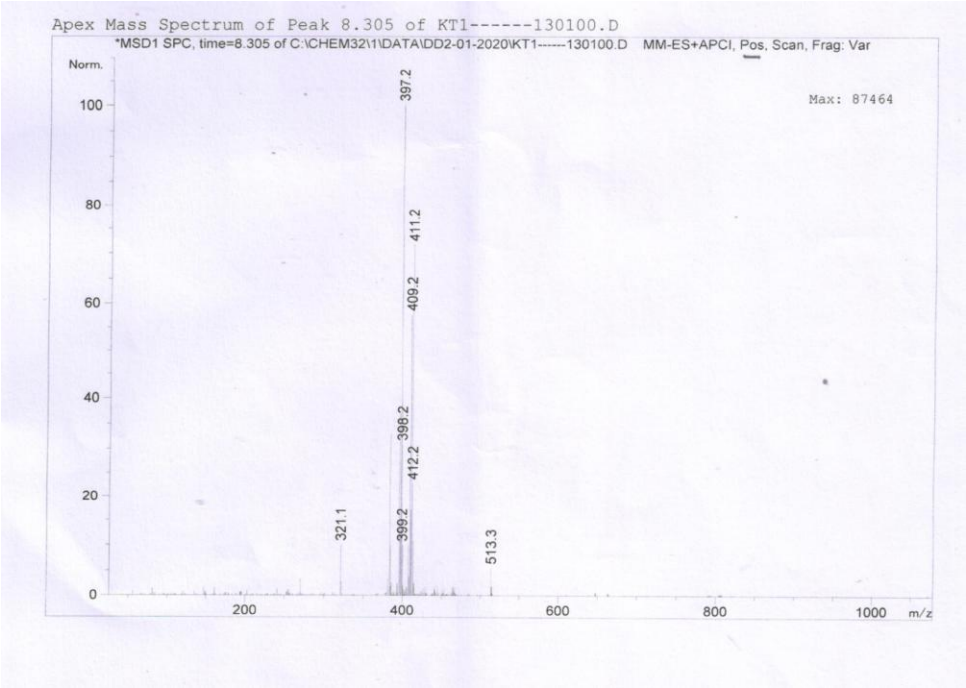



**Fig no 12: Mass spectrum of isolated compound (Positive)**

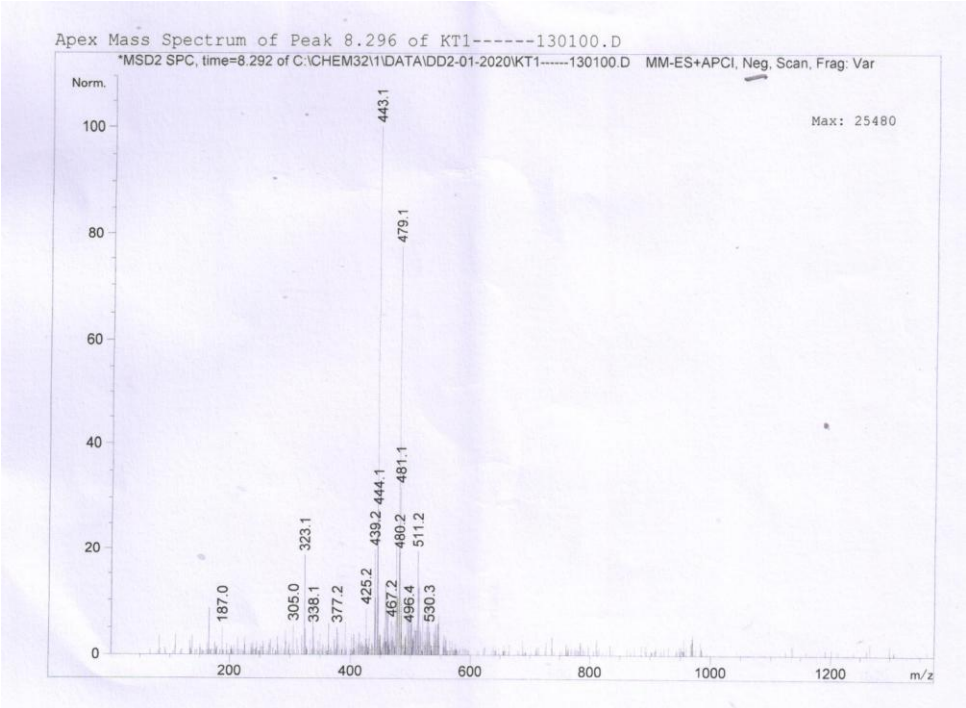



**Fig no 13: Mass spectrum of isolated compound (Negative)**

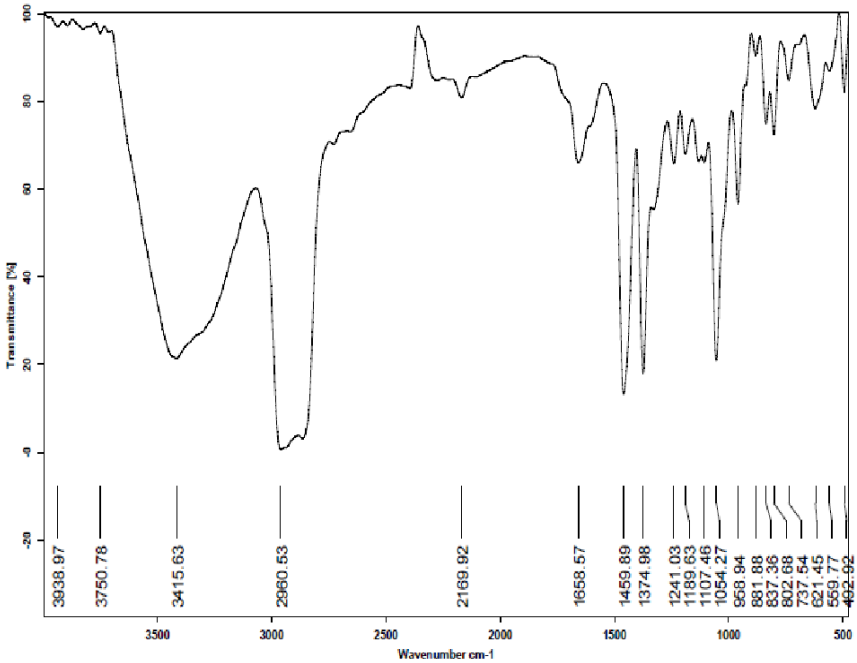



**Fig no 14: IR spectra of isolated compound**

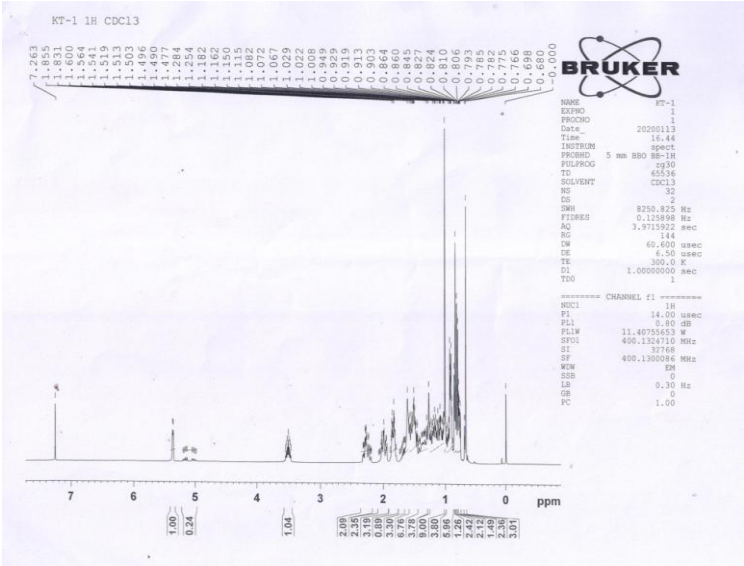



**Fig no 15: $^{1}$H NMR Spectra of Isolated Constituent**

There was no significant difference in bulk densities, tapped densities of prepared granules. Hausnerratio was found in the range of 1.05-1.14. Since Hausnerratios were below 1.24 indicates good flow property of granules. Granules of almost all the formulations gave a compressibility index ranged from 6.55-14.21% and angle of repose in the range of 22.15º - 28.56º. A compressibility index of greater than 25% is considered to be an indication of poor flowabilityand below 15% an indication of excellent flow ability of granules. Since all the formulations had a Carr's index below 15% and angle of repose below 30º granules possess good flow property.

**Table no 6:** Evaluation of β-sitosterol granules

| **Formulation Code** | **Evaluation Parameters** | | | | |
|---|---|---|---|---|---|
| | **Bulk Density (g/ml)** | **Tapped Density (g/ml)** | **Hausner Ratio** | **Carr's Index (%)** | **Angle of Repose θ** |
| F1 | 0.81±0.02 | 0.78±0.02 | 1.05±0.06 | 6.55±0.05 | 28.56±0.05 |

| | | | | | |
|---|---|---|---|---|---|
| F2 | 0.78±0.05 | 0.76±0.02 | 1.08±0.02 | 8.02±0.02 | 23.21±0.06 |
| F3 | 0.76±0.02 | 0.74±0.02 | 1.16±0.03 | 12.42±0.05 | 22.15±0.03 |
| F4 | 0.75±0.03 | 0.72±0.02 | 1.14±0.02 | 14.21±0.04 | 23.52±0.05 |

**Table no 7: Physical charecterization of β-sitosterol capsule**

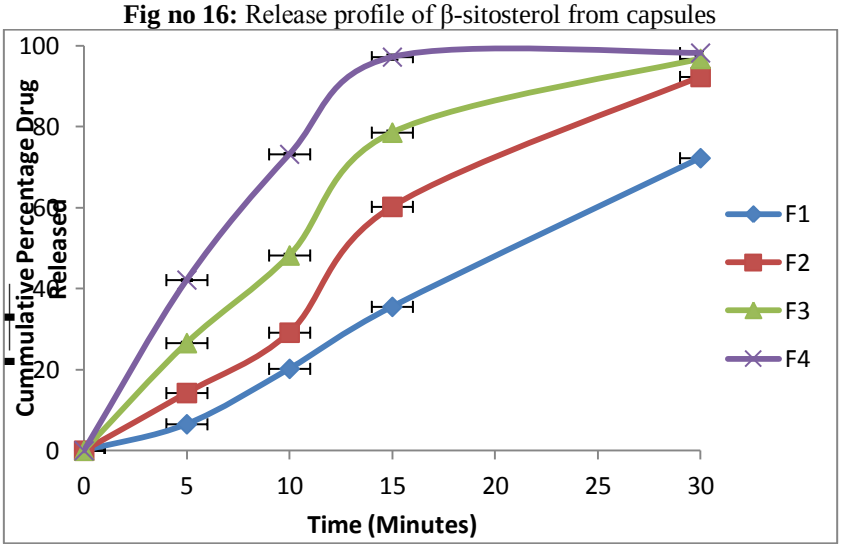
| **Formulation Code** | **Evaluation Parameters** | | |
|---|---|---|---|
| | **Weight variation (mg)** | **β-sitosterol content (%)** | **Disintegration Test (mins)** |
| F1 | 398±0.4 | 98.02±0.5 | 3.5±0.3 |
| F2 | 400±0.5 | 99.5±0.2 | 3.2±0.5 |
| F3 | 401±0.2 | 99.2±0.6 | 2.8±0.5 |
| F4 | 399±0.5 | 99.5±0.4 | 2.2±0.4 |

β-sitosterol capsule formulations pass the test for weight variation according to I.P 2010 since the percentage deviation of individual weight of capsule from mean were found within ±7.5%. Drug (β-sitosterol) content of all the capsule formulations were more than 85%. Disintegration time of formulations F1, F2, F3, F4 was found to be 3.5, 3.2, 2.8, 2.2mins respectively which indicate that as the concentration of SSG increased the rate of disintegration also increased**.**

**Table no 8: Dissolution Profile of β-Sitosterol capsules**

| **Time (Mins)** | **F1** | **F2** | **F3** | **F4** |
|---|---|---|---|---|
| 0 | 0±0 | 0±0 | 0±0 | 0±0 |
| 5 | 6.53±0.56 | 14.25±0.18 | 26.58±0.34 | 42.15±0.23 |
| 10 | 20.22±0.42 | 29.15±0.34 | 48.22±0.45 | 73.21±0.34 |
| 15 | 35.53±0.21 | 60.23±0.21 | 78.56±0.53 | 97.21±0.65 |
| 30 | 72.25±0.56 | 92.32±.43 | 96.78±0.75 | 98.23±0.43 |

**Fig no 16:** Release profile of β-sitosterol from capsules




It is observed that with increasing the concentration of sodium starch glycolate, the rate and extent of drug release from the formulation F2, F3, F4 were also increased. Thus, the release characteristics were significantly influenced by the concentration of super disintegrants used. The formulation F4 is selected as a better formulation for further studies.

**Table no 9: Effect of formulation on Total number of feces in castor oil induced diarrhea in rats.**

| Treatment group | Total number of feces | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | R1 | R2 | R3 | R4 | R5 | R6 | Mean | SD | SEM |
| Saline (2 ml/kg) | 17 | 18 | 16 | 17 | 14 | 18 | 16.67 | 1.51 | 0.61 |
| Loperamide (5 mg/kg) | 7 | 7 | 8 | 8 | 6 | 7 | 7.17 | 0.75 | 0.31 |
| Formulation (5 mg/kg) | 15 | 14 | 13 | 14 | 12 | 14 | 13.67 | 1.03 | 0.42 |
| Formulation (50 mg/kg) | 12 | 11 | 10 | 11 | 11 | 12 | 11.17 | 0.75 | 0.31 |

**Table no 10: Effect of formulation on Total number of diarrheal feces in castor oil induced diarrhea in rats.**

| Treatment group | Total number of diarrheal feces | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | R1 | R2 | R3 | R4 | R5 | R6 | Mean | SD | SEM |
| Saline (2 ml/kg) | 14 | 13 | 12 | 15 | 11 | 14 | 13.17 | 1.47 | 0.60 |
| Loperamide (5 mg/kg) | 4 | 5 | 7 | 7 | 6 | 6 | 5.83 | 1.17 | 0.48 |
| Formulation (5 mg/kg) | 11 | 12 | 10 | 12 | 9 | 10 | 10.67 | 1.21 | 0.49 |
| Formulation (50 mg/kg) | 9 | 8 | 10 | 8 | 6 | 7 | 8.00 | 1.41 | 0.58 |

**Table no 11:** Effect of formulation on Volume of intestinal content (ml) of diarrheal feces in castor oil induced diarrhea in rats

| Treatment group | Volume of intestinal content (ml) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | R1 | R2 | R3 | R4 | R5 | R6 | Mean | SD | SEM |
| Saline (2 ml/kg) | 3.5 | 4 | 4.5 | 3.5 | 3 | 3.5 | 3.67 | 0.52 | 0.21 |
| Loperamide (5 mg/kg) | 1.2 | 1.4 | 1 | 2.2 | 1.2 | 1 | 1.33 | 0.45 | 0.18 |
| Formulation (5 mg/kg) | 3.2 | 2.8 | 2.6 | 3.1 | 2.6 | 2.3 | 2.77 | 0.34 | 0.14 |
| Formulation (50 mg/kg) | 1.8 | 2.2 | 2.3 | 2 | 2.3 | 1.5 | 2.02 | 0.32 | 0.13 |

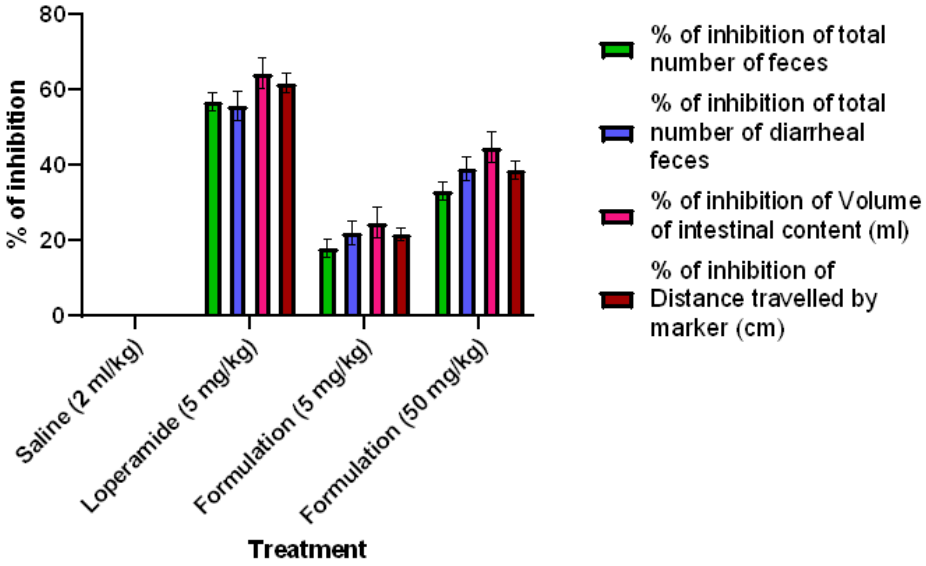
**Table no 12:** Effect of formulation on small intestinal transit in castor oil induced diarrhea in rats

| Treatment group | Distance travelled by marker (cm) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | R1 | R2 | R3 | R4 | R5 | R6 | Mean | SD | SEM |
| Saline (2 ml/kg) | 85 | 76 | 75 | 81 | 69 | 79 | 77.50 | 5.50 | 2.25 |
| Loperamide (5 mg/kg) | 28 | 29 | 28 | 23 | 34 | 35 | 29.50 | 4.42 | 1.80 |
| Formulation (5 mg/kg) | 65 | 66 | 58 | 54 | 53 | 68 | 60.67 | 6.50 | 2.65 |
| Formulation (50 mg/kg) | 48 | 47 | 49 | 46 | 41 | 54 | 47.50 | 4.23 | 1.73 |

**Table no 13:** Effect of formulation on castor oil induced diarrhea in rats

| Treatment group | % of inhibition of total number of feces | % of inhibition of total number of diarrheal feces | % of inhibition of Volume of intestinal content (ml) | % of inhibition of Distance travelled by marker (cm) |
|---|---|---|---|---|
| Saline (2 ml/kg) | -- | -- | -- | -- |
| Loperamide (5 mg/kg) | 56.9 | 55.7 | 64.5 | 61.9 |
| Formulation (5 mg/kg) | 17.9 | 22.07 | 24.7 | 21.7 |
| Formulation (50 mg/kg) | 33.1 | 39.2 | 44.9 | 38.7 |

**Fig no 17: Effect of formulation on castor oil induced diarrhea in rats**




**Castor oil induced diarrhea**

In castor oil induced diarrhea test, formulation showed considerable antidiarrheal effect in rats. At the dose 50mg/kg of formulation exhibited significantly inhibited the frequency of defecation when compared with untreated control rats ($P<0.05$). Formulation treated rats showed decreased the total number of wet feces produced upon administration of castor oil when compared to the castor oil treated rats.

**Castor oil induced enteropooling**

Formulation showed noticeable effect in castoroil induced entropooling test in the rats. The formulation (50 mg/kg bd.wt.) treated rats showed intestinal volume was decreased by 44.9% for and values were statistically significant ($P<0.05$). The standard drug, loperamide (5 mg/kg), also significantly inhibited intestinal fluid accumulation (64.5%) ($P<0.01$) and the effect of the formulation was less potent in comparison to the standard drug.

**Gastrointestinal motility test**

The gastrointestinal distance traveled by the charcoal meal in the rats significantly ($P<0.01$) (38.7%) lessened by formulation (50 mg/kg bd.wt.) treated rats compared with the control group. Loperamide (5 mg/kg) produced a marked decrease (61.9%) in the propulsion of charcoal meal through gastrointestinal tract.

## IV. Discussion

Diarrhea is usually considered a result of altered motility and fluid accumulation within the intestinal tract. The objective of diarrheal test is to determine the effect of beta sitosterol formulation on castor oil induced diarrhea. Castor oil is a triglyceride characterized by a high content of the hydroxylated unsaturated fatty acid ricinoleic acid[20]. About 90% of ricinoleate present in castor oil is mainly responsible for diarrhea

After oral ingestion of castor oil, ricinoleic acid is released by lipases in the intestinal lumen, and considerable amounts of ricinoleic acid are absorbed in the intestine. Presence of ricinoleate in small intestine, the peristaltic activity of small intestine increases as a result of permeability of Na+ and Cl- changed in the intestinal mucosa[21]. Secretion of endogenous prostaglandin is also stimulated by ricinoleate.

Prostaglandins of the E series are considered to be good diarrheogenic agents in experimental animals as well as in human beings. The inhibitors of prostaglandins biosynthesis are therefore considered to delay castor oil-induced diarrhea. Prostaglandins are associated with changes in the bowel that stimulate diarrhea. Recent study shows that the laxative effect of ricinoleic acid present In castor oil is due to the induction of contraction of intestinal smooth muscle which is mediated by activation of EP3 receptors on intestinal smooth-muscle. Many anti-diarrheal agents act by reducing the gastrointestinal motility and/or the secretions[22]. Inhibitors of prostaglandin biosynthesis delay castor oil induced diarrhea.. Pharmacological screening of β-sitosterol revealed various activities like antimicrobial, anti-Inflammatory, anticancer, antifertility, angiogenic, antioxidant, immunomodulatory, antidiabetic, antinociceptive without major toxicity. From the result, we found the our formulation suppressed the propulsion of charcoal meal by increasing the absorption of water and electrolytes due to their PG inhibiton and anti mycobacterial properties.

## V. Conclusion

The present studies are being performed pharmacognostical evaluation of *Alocasia fornicata* Leaves, isolate the compound named as the beta sitosterol based on Analytical evidence.

Developed novel β-Sitosterol derivative formulation and evaluate the possible anti-diarrhoeal properties of the Formulation. Developed formulation was evaluated for castor oil induced diarrhoea and enterpooling as well as intestinal transit in rats.

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