

**FORMULATION AND EVALUATION OF HERBAL TABLET CONTAINING METHANOLIC EXTRACT OF *CALOPHYLLUM INOPHYLLUM***

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***Corresponding author e-mail:** drusmishra@rediffmail.com**ABSTRACT**

The present paper deals with formulation and evaluation of herbal tablets prepared from methanol extract of the selected plant. A solid pharmaceutical dosage formulation using a novel dry plant extract (stem barks) using various excipients viz., sprays dried lactose, starch 1500, and Aerosil-200 and magnesium stearate by direct compression method. The absorption curve of *Calophyllum inophyllum* Methanolic extract showed characteristic absorption maximum at 278 nm in 0.1N HCl. The drug obeyed Beer's law in the concentration range of 10mcg/ml to 180mcg/ml, and it was found to be linear with $r^2 = 0.999$, regression equation $Y = 0.013x + 0.005$. It was found that the release rate of drug increased as the percentage of starch 1500 was increased from 10 mg to 30mg. As the concentration of starch 1500 increased the release rate increased from 67.49% to 99.35% (CIT4) in 6 hours by increasing the concentration of starch 1500. The optimized formulation CIT4 of the drug was subjected to accelerated stability studies and the results were reproducible, even on tablets that had been stored for about 3 months at 25°C/60% RH, 30°C/60% RH and 40°C/75% RH.

Keywords: *Calophyllum inophyllum*, Methanolic extract, Herbal tablet formulations, Aerosil and magnesium stearate

INTRODUCTION

Plants are always an exemplary source of drug. In fact, many of the currently available drugs were derived either directly or indirectly from the plants. The plant kingdom represents a rich source of organic compounds, many of which have been used for medicinal and other purposes¹.

Herbal medicine remains the major source of health care for the world's population. *Calophyllum inophyllum* belongs to family Clusiaceae (syn. Guttiferae) is a medium sized to large evergreen tree that average 8-20m in height with a broad spreading crown of irregular branches². In India it is distributed in the coastal regions of Maharashtra, Karnataka, Kerala, Tamil Nadu, Andhra Pradesh, Orissa, and the Andamans; often it runs wild; also reported to be growing in Arunachal Pradesh. About seven species occur in India. Some species are ornamental and

others yield timber, commercially classified as POON and oil³.

In different parts of India, the plant is known by different vernacular names /local names are English - Alexandra Laurel, Alexandrian Laurel, Hindi - Sultanachampa, surpunka, undi, Sanskrit - Nagachampa, punnaga, surangi, Oriya - Polang, ponnang, Tamil- Pinnai, punnagam^{2,3}. As per the ethnomedicinal information the various parts of *Calophyllum inophyllum* possess medicinal properties. The fresh bark of *C. inophyllum* is used to treat diabetes⁴. The fresh fruit and its oil used externally against rheumatism, in topical infection and seborrhea in human adult^{5,6}. The dried leaf and its decoction used to cure rheumatism, skin-infections⁷, cuts and sores⁸⁻¹⁰. The fresh leaves infusion are used to cure bacterial infection, fungal infection and as vermifuge/ pediculicide⁶. The resin are used orally as an emetic and purgative¹¹. Dried

seed extract are used against rheumatism in human adult¹². In Java the trees are supposed to possess diuretic properties^{2, 3}. The stem barks possess antibacterial and analgesic activities¹³.

The reported chemical constituents present in *C. inophyllum* are flavonoid compound Amentoflavone^{14,15}, Steroid compound campesterol^{16,17}, Arachidic acid lipid¹⁷, xanthone derivative Brasilixanthone-B and Buchanaxanthone^{18,19}, coumarin derivatives Calocoumarin-A, Calocoumarin-B, Calocoumarin-C and Apetalolide²⁰ and Beta Amyrin a triterpene.

Before the extract subjected for the formulation preparation, the chloroform and methanol extracts of *Calophyllum inophyllum* stem barks were evaluated for their analgesic and anti-inflammatory activity studies by using different animal models like hot plate method, tail immersion method, acetic acid method, Carrageenan induced rat paw edema method, Complete Freund's Adjuvant (CFA) method and compared with the Standard drugs i.e. Morphine sulphate (5mg/kg), Diclofenac Sodium (5mg/kg) and Indomethacin 4mg/kg respectively. Among the two extracts the methanol extract at the dose of 200mg/kg body weight showed significant biological activities as compared to standard drug. The dose of the extract was selected for biological activity based on acute toxicity studies. The experimental protocols were cleared by Institutional Animal Ethical Committee, Royal College of Pharmacy and Health Sciences, Berhampur (Vide No.10/2008/CPCSEA, dt.20.03.2008). Based on the analgesic and anti-inflammatory activities results, in the present study we design to formulate and evaluate the herbal tablet formulations containing methanol extract of *Calophyllum inophyllum* stem barks. The drug concentration used in the formulation was calculated on the basis of their drug tolerance study and effective dose on animal model. In tablet formulation the drug content per tablet was 100mg.

MATERIALS AND METHODS

Plant material: The stem barks of *Calophyllum inophyllum* were collected from the forest of Similipal Biosphere Reserve, Mayurbhanj, Orissa in August 2006. The plant material was identified and authenticated taxonomically at the Central National Herbarium, Botanical Survey of India, Botanical Garden, Howrah-711103, West Bengal, India (Ref no-CNH/I-I(59)/2006/Tech II, dated- 27.10.2006). A voucher specimen of the collected sample was deposited in the institutional herbarium for future reference.

Preparation of extracts: The collected stem barks were cleaned, dried under shade and powdered by a mechanical grinder. Hundred grams of the pulverized stem bark was extracted with petroleum ether, chloroform and methanol successively in a soxhlet apparatus. Petroleum ether was used in initial step of extraction for defatting the plant materials. The successive extracts were separately filtered and concentrated at reduced temperature on a rotary evaporator. The yield was found to be around 2.52; 4.81 and 21.36% (W/W) respectively. The biologically potent methanol extract was prepared for herbal tablet formulation.

Powder Characteristics: Herbal powders are of wide range with varied physical properties and micromeritic properties. Powdered solids are heterogenous because they are composed of individual particles of widely differing sizes and shapes randomly interspersed with air spaces. It is more complicated in case of herbal powders to convert into tablet.

Angle of repose: Flow properties of the physical mixtures of all the formulations were determined by calculating angle of repose by fixed height method. A funnel with 10 mm inner diameter of stem was fixed at a height of 2 cm. over the platform. About 10 gm of sample was slowly passed along the wall of the funnel till the tip of the pile formed and touches the stem of the funnel. A rough circle was drawn around the pile base and the radius of the powder cone was measured.

Angle of repose was calculated from the average radius using the following formula.

$$\theta = \tan^{-1} (h/r)$$

Where,

θ = Angle of repose

h = Height of the pile

r = Average radius of the powder cone

Bulk density: Bulk densities of all types of granules were determined by pouring gently 25 gm of sample through a glass funnel into a 100 ml graduated cylinder. The volumes occupied by the sample were recorded.

Tapped density: Tapped densities of all types of granules were determined by pouring gently 25 gm of sample through a glass funnel into a 100 ml graduated cylinder. The cylinder was tapped from height of 2 inches until a constant volume was obtained. Volume occupied by the sample after tapping were recorded and tapped density was calculated.

Compressibility: It is also one of the simple methods to evaluate flow property of powder by comparing the bulk density and tapped density. A useful empirical guide is given by the Carr's compressibility.

Hausner's ratio: It provides an indication of the degree of densification which could result from vibration of the feed hopper.

$$\text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Lower Hausner ratio – Better flow ability

Higher Hausner ratio – Poor flow ability

Preparation of herbal tablets from Calophyllum inophyllum extract: Herbal tablets were prepared separately by direct compression process using different proportions of spray dried lactose and starch 1500 and denoted as CIT 1, CIT 2, CIT 3 and CIT 4. The composition of various formulations is given in Table 1. All the ingredients were passed through mesh no. 100 and mixed with 1% aerosil (Aerosil-200) and 1% of magnesium stearate. The micromeritic properties were determined for all the mixtures. The powder mixtures possess good flow properties and good packing ability. Thus, the mixtures were directly compressible. Tablets were compressed each of 300 mg weight on a 10-station Mini Press-I rotary tablet compression machine fitted with 8-mm flat-shaped punches. No manufacturing defects were observed in tablets like capping, lamination and chipping.

EVALUATION OF HERBAL TABLET

Drug content uniformity test: From each batch 20 tablets were taken, weighed and finely triturated. An adequate amount of this powder equivalent to 100 mg of the drug was accurately weighed and shaken with 150 ml of 0.1N HCl for 10 minutes. The mixture was diluted with 0.1N HCl to produce 200ml and filtered. 10 ml of the filtrate was diluted to 100 ml with distilled water and the absorbance was measured at respective maximum wave length. The drug content in the formulation was calculated using the standard curve.

Hardness and friability test: Hardness was determined by using a Monsanto tablet hardness tester (n = 6). The friability of the tablets was tested using Roche friabilator.

Weight variation test: The tablets were evaluated as per I.P., 1996 for weight variation (n = 20) using 1mg sensitivity balance.

In vitro drug release: Drug release was assessed by dissolution test under the following conditions: n = 6(in triplicate), USP type II dissolution apparatus (Lab India, DISSO 2000) at 50 rpm in 900 ml of 0.1N HCl maintained at $37 \pm 0.5^\circ\text{C}$. The tablet was allowed to sink to the bottom of the flask before stirring. Special precaution was taken not to form air pockets on the surface of the tablet. Five milliliters of the sample was withdrawn by using a syringe filter at regular intervals and replaced with the same volume of pre warmed ($37 \pm 0.5^\circ\text{C}$) fresh dissolution medium. The drug content in each sample was analyzed after suitable dilution using UV spectrophotometer method at respective maximum wave length.

Drug Polymer Compatibility Studies: The interaction studies were carried out to ascertain any kind of chemical interaction of drug with the excipients used in the preparation of tablet formulations. Fourier-transform infrared (DRS) spectra were obtained by using an FT IR-Affinity-1 spectrophotometer (DRS-8000) SHIMADZU, Japan.

The dried pure drug sample CIP was previously ground and mixed thoroughly with potassium bromide, an infrared transparent matrix, at 1:5 (Sample: KBr) ratio, respectively. The KBr powder was used as blank for background correction in FT-IR (DRS) studies. Forty five scans were obtained at a resolution of 4 cm^{-1} , from $4000\text{ to }300\text{ cm}^{-1}$.

Stability testing of optimized herbal tablet formulation: The optimized formulation of the drug was subjected to accelerated stability studies at specified conditions of temperature and relative humidity of $25^\circ\text{C}/60\% \text{ RH}$, $30^\circ\text{C}/60\% \text{ RH}$ and $40^\circ\text{C}/75\% \text{ RH}$ for 3 months.

Development of UV-VIS Spectrophotometric method for estimation of formulated Herbal Tablet Scanning and determination of maximum wavelength (λ_{max}): In order to ascertain the wavelength of maximum absorption of the extract, different concentrations of the extract (10 $\mu\text{g}/\text{ml}$, 20 $\mu\text{g}/\text{ml}$ and 30 $\mu\text{g}/\text{ml}$) in 0.1 N HCl were scanned using spectrophotometer within the wavelength range of 400-200 nm against 0.1 N HCl as blank and the wavelength corresponding to maximum absorbance was noted.

Preparation of standard stock solution: Accurately weighed 100mg of extract was dissolved in 3ml of methanol in 100ml volumetric flask and volume was made up to the mark with 0.1 N HCl to give a clear solution of 1000µg/ml concentration.

Preparation of working standard solutions and construction of Calibration Curve: A series of different concentrations of extract were prepared from working stock solution. 0.1, 0.2, 0.3, 0.4, 0.5, 0.6.....1.8, 1.9 and 2.0 ml solutions were pipetted out from the working stock solution and were transferred in to 10 ml volumetric flasks. 10,20,30,40 upto 200µg/ml solutions were obtained respectively on making up the solution to 10 ml with 0.1 N HCl. The absorbances of all these solutions were measured against a blank at respective λ_{max} using a UV double beam spectrophotometer (UV/Vis-1700, Shimadzu, Japan). A standard plot of absorbance v/s concentration of extract gives the standard calibration curve of the extract. This curve was used to determine in vitro drug release and drug content of herbal tablets and the observation is given in Table 4.

RESULTS AND DISCUSSION

The various composition of the prepared herbal tablet formulations are shown in Table 1. The micromeritic properties were determined for all the physical mixtures of *Calophyllum inophyllum*. The results of angle of repose, Carr's Index and Hausner ratio indicated that the powder mixtures possess good flow properties and good packing ability. The physical properties of tablet were determined and the results of the uniformity of weight, hardness, drug content and friability of the tablets are given in Tables 3. All the samples of the test product complied with the official requirements of uniformity of weight. The drug content was found to be close to 100% in all formulations. The low friability indicates that the herbal tablets are compact and hard. The results are reproducible, even on tablets that had been stored for 3 months at 25 °C and 60% relative humidity. The absorption curve of *Calophyllum inophyllum*

Methanolic extract showed characteristic absorption maximum at 278 nm in 0.1N HCl. The drug obeyed Beer's law in the concentration range of 10mcg/ml to 180mcg/ml, and it was found to be linear with $r^2 = 0.999$, regression equation $Y = 0.013x + 0.005$. In-vitro dissolution studies were conducted on tablets of each of the formulations such as CIT1, CIT2, CIT3 and CIT4. The mean cumulative percent of drug released at different time intervals for each formulation is shown in Table 4 and Fig.1. It was found that the release rate of drug increased as the concentration of starch 1500 increased. In formulations containing dried Methanolic extract of *Calophyllum inophyllum*, as the herbal drug was converted to tablet formulation, the release rate was reduced from 98.18% (CIP) to 67.49% (CIT1) in 6 hours. The release rate increased as the percentage of starch 1500 was increased from 10 mg to 30mg. As the concentration of starch 1500 increased the release rate increased from 67.49% to 99.35% (CIT4) in 6 hours by increasing the concentration of starch 1500. The FT-IR spectra of tablet formulations did not show the presence of any additional peaks for new functional groups. The major peaks of the drug remained unchanged in the mixtures. These results suggest absence of any chemical interaction between the drug (CIP) and the excipients used in tablet formulations. Hence, the drug was found to be compatible with all the excipients used. The optimized formulated herbal tablet CIT-4 was kept for stability studies at different temperature and relative humidity conditions to ascertain the stability of the drug. The results were reproducible, even on tablets that had been stored for about 3 months at 25°C/60% RH, 30°C/60% RH and 40°C/75% RH.

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Table 1: Composition of various tablet formulations containing *Calophyllum inophyllum* Methanolic extract

Formulation Code	Drug (mg)	Spray Dried Lactose (mg)	Starch 1500 (mg)	Mg. Stearate (mg)	Aerosil (mg)	Total Wt. (mg)
CIT1	100	184	10	3	3	300
CIT 2	100	179	15	3	3	300
CIT 3	100	174	20	3	3	300
CIT 4	100	164	30	3	3	300

CIT = *Calophyllum inophyllum* Methanolic extract tablet formulation

Table 2: Micromeritic parameters of physical mixtures containing *Calophyllum inophyllum* Methanolic extract

Formulations	Bulk density (gm/ml)	Tapped density (gm/ml)	% Compressibility	Hausner ratio	Angle of repose
CIT 1	0.52	0.68	23.52	1.3	28.56
CIT 2	0.53	0.71	25.35	1.33	30.15
CIT 3	0.55	0.74	25.67	1.34	30.54
CIT 4	0.58	0.79	26.58	1.36	32.54

CIT = *Calophyllum inophyllum* Methanolic extract tablet formulation**Table 3: Physical properties of compressed tablets of *Calophyllum inophyllum* Methanolic extract**

Formulations	CIT 1	CIT 2	CIT 3	CIT 4
Drug content (mg/tab)	102.7±1.8	101.2±0.6	98.1±1.5	99.1±1.9
Weight variation (%)	±5.5	±5.6	±5.4	±5.1
Hardness(kg/cm ²)	5.1±0.4	5.1±0.4	5.2±0.6	5.3±0.7
Friability (%)	0.96	0.78	0.95	0.87

CIT = *Calophyllum inophyllum* Methanolic extract tablet formulation**Table 4: In-vitro dissolution profile of herbal tablet containing *Calophyllum inophyllum* extract in 0.1 N HCl**

Mean cumulative percent ± S.D* drug released					
Time (hr)	CIP	CIT 1	CIT 2	CIT 3	CIT 4
1	24.26±3.56	18.74 ± 2.61	27.894±2.54	25.87 ± 3.39	23.34±3.43
2	33.31 ± 2.98	28.24 ± 2.69	42.373±2.98	36.06 ± 3.30	32.23±3.21
3	62.06 ± 3.91	39.36 ± 2.76	52.755±2.78	53.39 ± 3.57	58.76±3.76
4	87.32 ± 2.44	50.99 ± 3.69	62.260±2.87	67.14 ± 3.77	81.34±3.22
5	98.18 ± 3.52	59.18 ± 2.85	71.189±3.34	73.86 ± 3.62	97.98±3.21
6	98.18 ± 3.54	67.49 ± 2.72	75.973±3.23	78.48 ± 3.09	99.35 ± 2.31

* SD values (n=6), CIP= *Calophyllum inophyllum* pure Methanolic extract**Table 5: Stability data of the optimized formulation CIT- 4**

Time	% Drug content at different storage conditions		
	25 ⁰ C/60% RH	30 ⁰ C/60% RH	40 ⁰ C/75% RH
1 month	99.71	99.58	99.44
2 months	99.50	98.36	98.62
3 months	99.37	98.23	97.39

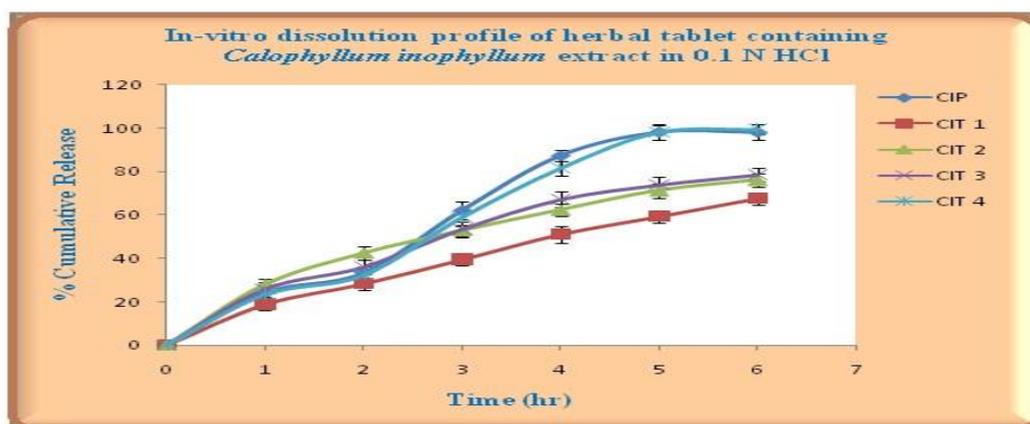


Fig 1: In-vitro dissolution profile of *Calophyllum inophyllum* extract from the formulations in 0.1 N HCl

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