

"Formulation and Evalution of Harbal Ointment by Using Phyllanthus Amarus Extract"

¹Ankit P. Gondane ²Chetan J. Gautam, ³Paresh N. Shivhare, ⁴Abhay D. Lilhare, ⁵Pankaj R. Khobragade

Manoharbhai Patel Intsitute of Bachelor of Pharmacy, Kudwa Gondia *Herbal and Drug Technology Corresponding Author: Ankit P. Gondane

Submitted: 15-04-2024

Accepted: 25-04-2024

ABSTRACT: Phyllanthus amarus Schum. & Thonn, belongs to the family Euphorbiaceae is a small herb well known for its medicinal properties and widely used worldwide. P. amarus is an important plant of the Indian Ayurvedic system of medicine which is used in the problems of stomach, genitourinary system, liver, kidney and spleen. It is bitter, astringent, stomachic, diuretic,febrifuge and antiseptic. extract of Phyllanthus amarus, was obtained by Soxhlet apparatus of I part of pulverized dried leaves in 4 parts of methanol. Simple ointment IP was used as ointment base for dispersion of the plant extract Parameters investigated include, spreadability, pH and viscosity of extract and formulated ointment while streptomycin antibiotics were used as controls. The result shows dried leave part of plant powdered and extracted by hot-continuous technique of extraction with ethanol. The pH of the ointment was $8.32 \pm$ 8.68 and the spreadability was 62 ± 70 mm. slightly washable, and nonirritant viscous in nature, soluble in ether and alcohol, no pink colour formed the Rancidity test are passed and loss on draying is passed. The formulated herbal ointment have significant properties and hence will be better, safe than allopathic and effective medications. Phyllanthusamarus ointment of the crude methanol extract has good therapeutic values for combating topical infections diseases. The phyllanthusamarus ointment is used as a potential agent for the treatment of wounds and skin infections.

KEYWORDS: Herbal ointment, phyllanthus amarus, microorganism, applied on wound.

I. INTRODUCTION

A **herb** is a plant or part of a plant used for its Scent, flavor of therapeutic properties. Herbal medicine is related to the study or practice of the medicinal and therapeátic uses of plants^[1] **Herbal medicine** has its origins in ancient cultures. It involves the medicinal use of plants to treat disease and enhance general health and wellbeing.Some herbs have potent (powerful) ingredients and should be taken with the same level of caution as pharmaceutical medications.^[2]

Phyllanthus amarus is also known as "Bhui Amla" and is belonging to the family phyllanthaceae. Phyllanthus amarus Schum & Thonn belongs to the family Euphorbiaceae is a small herb well known for its medicinal properties and widely used worldwide P. amarusis an important plant of Indian Ayurvedic system of medicine which is used in the problems of stomach, genitourinary system, liver, kidney and spleen. It is bitter, astringent, stomachic, diuretic, febrifuge, and antiseptic. The whole plant is used in gonorrhea, menorrhagia, and other genital affections. It is useful in gastropathy, diarrhea, dysentery, intermittent fevers, ophthalmopathy, scabies, ulcers, and wounds.^[6]

Phyllanthusamarus is also known as "Bhui and is belonging to the family Amla" phyllanthaceae. Phyllanthusamarus Schum & Thonn belongs to the family Euphorbiaceae is a small herb well known for its medicinal properties and widely used worldwide P. amarus is an important plant of Indian Ayurvedic system of medicine which is used in the problems of stomach, genitourinary system, liver, kidney and spleen. It is bitter, astringent, stomachic, diuretic, febrifuge, and antiseptic. The whole plant is used in gonorrhea, menorrhagia, and other genital affections. It is useful in gastropathy, diarrhea, dysentery, intermittent fevers, ophthalmopathy, scabies, ulcers, and wounds.^[6] An herbal ointment containing methanol extract of the aerial part of Phyllanthus amarus was formulated and parameters including antimicrobial properties against some human pathogens such as Bacillussubtilis, staphylococcus aureus, Escherichia coli,



pseudomonas aeruginosa, and aspergillusniger were evaluated.

Ointment

Ointments are preparations for external use, intended for application to the skin Typically, they have an oily or greasy consistency and can appear "stiff" as they are applied to the skin. Ointments contain drug that may act on the skin. Ointment is a smooth thick substance that is put on sore skin or a wound to help it heal. A range of ointments and creams is available for the treatment of eczema. He received ointment for his flaking skin Synonyms: salve, moisturizers, or cosmetics, can be applied to the eyes, skin, and mucus membranes to help treat anything from dry skin to cuts, scrapes, burns, bites, and hemorrhoids. An ointment is a homogeneous, viscous, semi-solid preparation, most commonly a greasy, thick oil (oil 80%- water 20%) with a high viscosity, that is intended for external application to the skin or mucous membranes.^[6]

Phyllanthus amarusointments:-

The phyllanthusamarus ointment are mainly used to treat skin ulcer and Inflammation.

Skin Ulcer :-

A skin ulcer is an open sore caused by poor blood flow.Good blood flow is necessary for wound healing. But if you have blood circulation problems, minor injuries can't heal properly. Over time, an injury can turn into a skin ulcer.

Generally, a skin ulcer looks like a round open sore in the skin.



Fig.1 Skin Ulcer^[4]

Inflammation:-

Inflammation is part of the body's defense mechanism. It is the process by which the immune system recognizes and removes harmful and foreign stimuli and begins the healing process. Inflammation can be either acute or chronic.

1) Acute Inflammation-Tissue damage due to trauma, microbial invasion, or noxious compounds can induce acute inflammation. It starts rapidly, Subacute inflammation is the period between acute and chronic inflammation and may last 2 to 6 weeks.



Fig.2 Inflammation^[5]

2) Chronic Inflammation-Chronic inflammation is also referred to as slow, long-term inflammation lasting for prolonged periods of several months to years.

Chronic inflammation can result from the following:-Failure of eliminating the agent causes an acute inflammation such as infectious organisms including Mycobacterium tuberculosis, protozoa, fungi, and other parasites that.

OBJECTIVE

- To collect the plant of Phyllanthusamarus. And studying the properties of plant.
- To formulate the ointment by using methanolic extract of Phyllanthusamarus.
- To evaluate the Parameters investigated include, spreadability, pH and viscosity of formulated ointment.
- Ointment is aimed to destroy or inhibit the growth of bacteria.

PLANT PROFILE

Plant name:-Phyllanthusamarus Synonyms:-

Odia Language -Bhumi amla, Bhuiaola.

Telugu Language- Nela Urika Hindi- Jangliamli, **Hindi** – Bhumi amla

English – Stone breaker, Shatter stone, Hurricane weed, Gulf leaf flower, Gale of wind, carry me seed, Black catnip

Sanskrit –Bhoodha free, Tamalakee,

Bhoomyaamlake

Bengali-Bhui amla

Tamil-Keelanelli (Keezhanelli)^[20]





Fig.3 plant of Phyllanthusamarus^[19]

Botanical characteristics:-P. amarus is an annual herb, glabrous and 10-60 cm tall. Its roots are woody and stout. Stems are angular and branched at base. Leaves are compound, sub – sessile, paripinnate, stipulate, and distichous. Leaflets are obscure and base rounded. Flowers are pedicellate, axillary and yellowish fruit is minute, globose, capsule and dehiscent. Fruit is present underneath the branches. The seeds are pale brown with parallel ribs on the back side and trigonous. The stalked capsule are 1-2mm long, smooth, round, 2 mm wide with sixseeds. P. amarus plant has explosive seeds that propel the seeds far from the plant^[20]

Plant parts Used: Stems, seeds, fruits, Leaves, and whole plants of P. amarus are used.

Biological Source:- It is a dry plant of PhyllanthusAmarus Belonging to the family Phyllanthaceae.

Geographical Source:-Phyllanthusamarus is a herbal plant found in tropical regions in India, China, Americas Abrica, South East Asia^{.[21]}

Taxonomical classification:-

- Kingdom- Plantae
- Division Angiospermae
- Class- Dicotyledoneae
- Order- Tubiflorae
- Family Euphorbiaceae
- Genus- Phyllanthus
- Species- amarus^[22]

Phytoconstituent of P. Amarus:-P. amarus is reported to contain many chemical constituents like lignans, phyllanthin, hypophyllanthin, uniphyllin and phyllniurin; Flavanone glycoside such as niranthin, nirtetralin, phyltetralin and lintetralin ; steroid hormone estradiol, triterpenes like phyllantheol, phyllanthenone, and phyllanthenol; flavonoids such as quercetin, quercitrin, and astragalin.^[20]

Topical uses of phyllanthusamarus:-

- The Phyllanthusamarus it shows is effective in relieving pain.
- ➤ The ointment is used to reduce pain & prolong treatment.
- ➤ The phyllanthusamarus extract ointments are used in skin infection eg: Rases,Cracking etc.
- ➤ The phyllanthusamarus are Used in as relieving pain of Knee Osteoarthritis.
- ➤ The topical use of P. amarus as a Poultice for Skin ulcers.
- ► It is used in sores, swelling and inflammation^{.[23]}

II. MATERIALS AND METHODS

1. Chemicals and reagent

The various chemicals used throughout experimental work are summarized.

Table N	Table No.1 Chemicals and Reagent			
Sr. No.	Chemicals			
1.	Phyllanthus amarus drug			
2.	Methanol			
3.	Wool fat			
4.	Cetostearyl alcohol			
5.	Hard paraffin			
6.	Soft paraffin			
7.	Sulphuric acid			

2. Instruments

The various instruments used throughout experimentation are given below.

Tuble 1002 Histi unients			
Sr. No.	Name of Instruments		
1.	Soxhlet apparatus		
2.	Heating mental		
3.	Distillation chamber		
4.	Incubator		
5.	Autoclave		
6.	Laminar air flow		

Table No.2 Instruments

3. Collection and drying of plant material

The powder of phyllanthusamarus collected locally from Gondia. The powder of phyllanthusamarus was stored in a well-stoppered container. The dried material of the plant is then used for further work.

EXTRACTION

1. Soxhlet extraction

Method of extraction, we have performed for separation of the medically active portion of the plant of phyllanthusamarus from the inactive or inert components. For the Extraction process, we



required the dried leaves of plant have phyllanthusamarus, solvent and soxhlet apparatus is a type of extractor laboratory apparatus for the Extraction of lipid and other molecules from a solid sample. A Soxhlet Extraction apparatus is composed of a condenser, a Soxhlet extractor, and round bottom flask A Soxhlet Extraction method is somewhat opposite from filtration in that the extraction of the sample is for an insoluble organic component in a solvent, unlike complete solubility of an organic component in a sample, which is suitable for just simple filtration. Therefore, a Soxhlet Extraction procedure allows acompound to be extracted from insoluble material using a In an Extraction process, we chemical solvent have to performed such following steps to obtain quality extract from phyllanthusamarus. In the first we collect the powder of phyllanthusamarus. Then in the next steps we performed Extraction, so Extraction we set up the Soxhlet for apparatuscondenser, round bottom flask and heater system, in the proper manner.



Fig.no.4 Soxhlet Apparatus

1. Preparation of ointment: -Formula for Simple Ointment: -

as per SOP.

The powder- of phyllanthusamarus can be in Soxhlet condenser in required quantity or may be upto 50 gm and in round bottom flask, we used the 250 ml of methanol solvent for the Extraction

The solvent methanol heated on 70° - 80° C continuously. Then the process takes time upto 4-5 hr. to bring Extract. The excess solvent was removed by the performing evaporation and distillation method and the further concentrated in a vacuum oven^[6]

4. Characteristics of extract

Characteristics of extract are given below in table. Percentage yield of P. amarus extract

	Table No.3	Characteristics	of	extract
--	------------	------------------------	----	---------

Sr.	Drug	Solvent	% Yield		
No.					
1.	Phyllanthus	Methanol	11.36%		
	amarus				



Fig.no.5 Weighing Machine (% Yield)

III. PREPARATION OF OINTMENT

Table No.4 Formulation of Ointment Standard Working F1 F2 F3 Sr. No. Ingredients Formula Formula 1.0 g 1.0 g Wool fat 10.0 g 1.0 g 1.0 g 1. Hard paraffin 2. 10.0 g 1.0 g 1.0 g 1.0 g 1.0 g 3. Cetostearyl 10.0 g 1.0 g 1.0 g 1.0 g 1.0 g alcohol 4. White Soft 16.0 g 60.0 g 16.5 g 16.0 g 15.5 g paraffin 5. Extract 10.0 g 1.0 g 0.5 g 1.0 g 1.5 g 20.0 g Total 100.0 g 20.0 g 6. 20.0 g 20.0 g

Procedure:- The preparation of simple ointment by fusion method.

Clean all the glassware and dry them properly

• Weigh all the ingredient properly.

• Take hard paraffin and cetostearyl alcohol melt them in porcelain dish and kept on water bath.



- To above melted mixture adds wool fat, white soft paraffin and extract then stir well.
- After melting all ingredients remove from porcelain dish.
- Pour it into an air tight container and place it in a cool and dry place.^[24]



Fig no. 6 Batch Formulation

IV. EVALUATION OF OINTMENT 1. Spreadability test:

Excess sample was placed between the two glass slides and 100 g weight was placed on the glass slide for 5 min to compress the sample to a uniform thickness. Weight (100 g) was added to the pan. The time in seconds required to separate the two slides was taken as a measure of spreadability.^[6]

Spreadability = 100gm 2.5cm/5

2. Washability test:

Washability was determined by rubbing the little amount of base on hand for test.

3. Irritancy test:

Small quantity of ointment applied on skin and wait for 10 minutes after 10 minutes we evaluate that ointment properties on skin.

4. Identification test Ointment:

- a. Colour
- b. Odour
- c. Consistency

5.Test of solubility:

The contents should be soluble in 9 parts of water and in 1.7 parts of hot water. The contents should be miscible with alcohol, ether and chloroform.^[25]

6. viscosity:

Viscosity of ointment was measured by the Brookfield viscometer. The correct spindle was selected (spindle no. 4) for the given product then the operating condition was setup. Then the viscosity was measured directly at 6rpm speed by keeping the torque constant. The mean was obtained. The viscosity is determined by following formula:^[26]

Viscosity=Dial Reading \times Factor, For. I.V-4 at 6 RPM Factor is 1M (1000)

7. Identification test of PH:

To find out the PH of Phyllanthus amarus ointment, in practical bases, the electrode can dip in the solution of Phyllanthus amarus. The solution was prepared by One gram of the weighed formulation was dispersed in 100 mL of diluted tween 80 (polysorbate 80).^[6]

8. Rancidity:

This test is performed by using the Phloroglucinol solution. The rancidity is due to the oxidation of the fats and oils; during oxidation free fatty ults are shown acids are liberated. These free fatty acids react with the Phloroglucinol solution and gives pink colour. This indicates the rancidity of the product.10 ml of melted ointment was taken then added 10 ml of concentrated hydrochloric acid and 10 ml of Phloroglucinol solution and shaken for one minute. The material shall be taken to have passed the test if no pink colour developed. ^[26]

9. Loss on draying:

Procedure:

- 1) Weigh about 1.5 g of the powdered drug into a weighed flat and thin porcelain dish.
- Dry in the oven at 100° c or 105° c, until two consecutive weighings do not differ by more than 0.5 mg.
- 3) Cool in a desiccators and weigh. The loss in weight is usually recorded as moisture.^[27]

V. RESULTS AND DISCCUSION 1. Preparation of Plant Extract

The dried leave part of plant powdered and extracted by hot-continuous technique of extraction with ethanol. And the solvent was removed by evaporation method with the help of water bath.

2. Evaluation Parameter of Herbal Ointment

In the evaluation parameter of herbal ointment, we perform different types of evaluation tests of ointment.

2.1 Physical appearance:-Colour, odour, thickness was studied.



Table No.5 Physical Appearance

Sr. No	Testa	Formulation		
Sr. No.	Tests	F1	F2	F3
1	Appearance	Light Green	Green	Dark Green
2	Odour	Aromatic	Aromatic	Aromatic
3	Consistency	Viscous, Greasy	Viscous, Greasy	Viscous, Greasy

2.2 Spreadability:-The spread ability test of Phyllanthus amarus ointment was found tobe-T-11 N.C.C.

Table No.6 Spreadability				
Sr. No	Teata	Formulation		
Sr. 10.	Tests	F1	F2	F3
1	Spreadability	62g.cmSec ⁻¹	65g.cmSec ⁻¹	70g.cmSec ⁻¹

2.3 Skin irritation test:-

The skin irritation test of given formulation of Phyllanthus amarus show no reaction-

	Skin	Formulation	Formulation		
Sr. No.	irritation test (Time)	F1	F2	F3	
1	10 min	No reaction	No reaction	No reaction	
	1 hour	No reaction	No reaction	No reaction	
	2 hour	No reaction	No reaction	No reaction	

Table No 7 Skin irritation test

2.4 pH OfOintment:-The pH of Phyllanthus amarus ointment was found to be-

Table No.8 pH of Ointment

Sn No	Tests	Formulation		
SI. NO.	1 6515	F1	F2	F3
1	pН	8.32	8.61	8.68

F1 pH F2 pH F3 pH



Fig. No.7 pH of Ointment

2.5 Viscosity:-The viscosity of the given ointment was found to be-

Table No.9 Viscosity of Ointment				
Sm No	Teata	Formulation		
Sr. No.	Tests	F1	F2	F3
1	Viscosity (cps)	38 cps	32 cps	30 cps



F1 Viscosity F2 Viscosity F3 Viscosity



Fig. No.8 Viscosity of Ointment

2.6 Washability:-	The washability test of ointment shows-
	Table No 10Washability test of Ointment

Table No.10 Washability test of Omtment				
Tests	Formulation			
Tests	F1	F2	F3	
Washability	Slightly washable and	Slightly washable and	Slightly washable and	
	Sticky	Sticky	Sticky	

2.7 Solubility:-

Table No. 11 solubility of Ointment

For Formulation F1; F2; F3.				
water	Alcohol	Ether	Chloroform	
Insoluble	Insoluble	Soluble	Soluble	
Fig.No.9 solubility of Ointment				

2.8 Rancidity:-

Table No.12 Rancidity of Ointment

Test	Formulation				
Rancidity	F1	F2	F3		
	No pink colour	No pink colour	No pink colour		
Fig. No.10 Rancidity of Ointment					

2.9 Loss on Draying: -

Table No.13 Loss on Draying						
Sr. No.	Tests	Formulation				
		F1	F2	F3		
1	Loss on drying	38%	13%	8%		





Fig. No.11 Loss on Draying

VI. CONCLUSIONS

Phyllanthus amarus ointment has good antimicrobial properties and can be used for the treatment of wounds and skin infection caused by susceptible organisms. The crude methanol extract of Phyllanthus amarus and Phyllanthus amarus formulated as-an-ointment extract have antibacterial activity against B. subtilis, S. aureus, E. coli, P. aeruginosa and A. niger. Our findings have justified the medicinal uses and speculations about the therapeutic values of this plant for combating topical infectious diseases. The activity against these organisms showed remarkable concentration dependence. The Phyllanthus amarus ointment can be used as a potential agent for the treatment of wounds and skin infections caused by these susceptible organisms.

From the present investigation, it has been revealed that herbal ointment of plant Phyllanthus amarus leaves extract can be formulated using white soft paraffin with other ingredients and the evaluation of physical parameters shown satisfactory results. Hence, from the overall results, finally it was concluded that the formulated herbal ointment have significant antimicrobial properties and hence will be better, safe and effective than allopathic medications.

REFERENCES

- [1]. https://effectivehealthcare.ahrq.gov/health -topics/herbal-medicine.
- [2]. https://www.betterhealth.vic.gov.au/health /conditionsandtreatments/herbal-medicine.
- [3]. https://www.healthline.com/health/skinulcer#symptoms.
- [4]. https://images.app.goo.gl/r58pXpZe27EU qDC57.
- [5]. https://images.app.goo.gl/Dj5n8eD9NSB Qc1sZA.
- [6]. John A. Avbunudiogba, The design and antimicrobial screening of herbal ointment of Phyllanthus amarus". IJOP, vol 4, 2014, Page no. 113-116.

- [7]. AdegokeAA, "Studies on phytochemical screening and antimicrobial potentials of Phyllanthus amarus against multi antibiotic-resistant bacteria" IJARINP, vol.3(3), 2010 Page no. 6-12
- [8]. JayaPrakashYadav, "Evaluation of Antimicrobial Activity of Synthesized Silver Nanoparticle Using Phyllanthus amarus and TinosporacordifoliMedicinal Plant" Nanomedicine and Nanotechnology, vol 5, 2014, Page no. 250
- [9]. Bunudiogba JA, "Development and evaluation of liquid oral phyto formulation of phyllanthusamarus." SciVerse ScienceDirect, 2013, Page no. 908-912
- [10]. AbhyankarG.Suprasanna,"Hairy root extract of Phyllanthus amarus induces apoptotic cell death in human breast cancer cells, IFSET Vol. 2, Page no. 526-532.
- [11]. Jala TO, "The Physicochemical, safety and antimicrobial properties of Phyllanthus amarus herbal cream and ointment." JOPI, Vol. 46, (2016), Page no. 169-178
- [12]. GhoshA. SharmaandTalukar G, "The relative protection Given by extract of phyllanthusamarus." Food and chemical toxicology, Vol. 30, 1992, Page no. 865-869
- [13]. JayRamPatel, "phyllanthusamarus: Ethnomedicinal use, phytochemistry and phytopharmacology. Journal of Ethnopharmacoloy, Vol. 138, 2011, Page no. 286-313
- [14]. MirjanaMS,MarijaM,DusicaM,"Phyllanph usiin D, an unusual hydrolysable tannin from Phyllanthus amrus." Phytochemistry, Vol. 31 (1992), pp 711-713
- [15]. OwolabiJ, MixW,"Antifungal and antibacterial activities of the ethanolic and aqueous extract of Kigelia



Africana(Bignoniaceae) stem bark. AJOB, Vol. 6 (14), 2007, Page no. 1677-1680,

- [16]. Oluwafemu Fand DebiriF, "The Antimicrobial Effect of Phyllanthus amarus and Parquetinanigrescens on Salmonellatyphi." AJOBR, Vol. 11, (2008), Page no. 215-219
- [17]. Oyewolroloyedaraoo. "Phytochemical antimicrobial and toxicity studies of whole plant extract of phyllanthusamarus." IJOB, 2013, Vol 02 (03), Page. No. 519-532
- [18]. Addai- menshahDonkoret, "Evaluation of Extracts From Sida acuta , Phyllanthusamarus, Parkiabiglobosa and their herbal ointment for therapeutic and biological activities" Heliyon 9, (2023) Page no. 343-348
- [19]. https://images.app.goo.gl/8bRAquNLSK W5eG7A6
- [20]. SunilKumarBisoyi, "Phytochemical, pharmacological potential and ethanomedical uses of Phyllanthus amarus" IJSR, Vol 07 (02), 2022, Page no.453-460.
- [21]. https://uses.plantnetproject.org/en/Phyllanthus_amarus_(PRO TA)#:~:text=Phyllanthus.
- [22]. https://en.m.wikipedia.org/wiki/Phyllanth us_amarus.
- [23]. https://www.peacehealth.org/medicaltopics/id/hn-2146004#:~:text=Phyllanthus.
- [24]. R.M. Mehta "Dispensing Pharmacy", PublicedBy Nirali Prakashan. Page no. 23.7
- [25]. https://pharmastate.academy/qc-tests-forointments-and-creams.
- [26]. Deore et al. "Formulation and Evaluation of ointment, Herbal Sunscreen, Pharmacognosy Journal, Vol 9, Issue 1, 2017, page no 235
- [27]. Dr. K. R. Khandelwal and Dr. Varunda Sethi. "Practical Pharmacognosy", Published by Nirali prakashan. Edition 54