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ANTIMICROBIAL ACTIVITY OF HERBAL OINTMENT BY USING ETHANOLIC EXTRACT OF PHYLLANTHUS AMARUS LEAVES.

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Abstract:

Objectives: *The aim of the current study was to evaluate the pharmacological activity of the ethanolic extract of phyllanthus amarus by using leaves for its antimicrobial activity against the clinically isolated human pathogens. The pathogens such as the Staphylococcus (gram positive bacteria) and E. coli (gram negative bacteria) were evaluated in the formulation of herbal ointment.*

Materials and methods:

The leaves are collected and then powdered, the bioactive compound was obtained by using the ethanol in the maceration process. Simple ointment base (B.P.) was prepared, then extract (Phyllanthus amarus) was incorporated to form a different concentration of semi-solid herbal ointment. Leaves extract was carried out , by the detection of phytochemical analysis and evaluation were done. In the antimicrobial study was done by the cup plate method by using the sensitivity of the organisms in ointment contains variety of concentrations of extract in the zone of inhibition.

Keywords: *Herbal Ointment, phyllanthus amarus , phytochemical analysis, antimicrobial activity.*

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INTRODUCTION:

Phyllanthus amarus Linn is a small annual herbaceous weed that grows 40 cm in height and it is locally known as Bhui Amalaki, belonging to the family of (Euphorbiaceae) [7]. It is widely found throughout the tropical and sub-tropical countries of the world. It is mostly used in the Indian Ayurvedic System of Medicine. Phyllanthus amarus is a plant that was reported of having a broad spectrum of pharmacological properties [6].

The important phytomolecules are alkaloids, flavonoids, tannins, terpenoids, and phenolic compounds. Due to their specialized biochemical capabilities plants are able to synthesize a vast array of primary and secondary phytochemical which are useful for humans[1]. Medicinal and aromatic plants are the vast source of potentially useful phytochemical for the development of new therapeutic agents or pharmaceutical products Phytochemical such as tannins and polyphenols from *Phyllanthus amarus* have been associated with some antimicrobial importance. Major Phytochemical and mineral contents are also reported on the whole plants. Many lignans were isolated from different parts of the plants such as phyllanthus and hypophyllanthin [2]. The highest amount of phyllanthus, hypophyllanthin and flavonoids such as gallocatechin, quercetin3-O-glucopyranoside, glucopyranoside, kaempferol has been also reported in leaves [8].

This small herbaceous weed also used in the folk medicine from ancient time ethno medicinal plant reported to have several biological properties such as antimicrobial and antiviral activities. It acts against hepatitis B and it is chemo-protective, anti-mutagenic and hypoglycemic agents as well. The plant parts also acts against cytotoxicity and improves the immune system of patients, too. It is also used as a tonic. It is used as diuretic and in the treatment of diabetes, Dysentery, hepatitis, and skin disorders[3]. It exhibited immuno-modulatory activity. The plant also contains several pharmacological important phyto molecules whose efficacy is well established by several biochemical and
Pharmacological studies. So from the previous studies it was found that this medicinal herb possesses huge ethno medicinal potentiality and considering those reports it was decided to investigate some important parameters of the
Experimental plant extracts.

***Phyllanthus amarus* (Figure .1)**

MATERIALS AND METHODS:

Collection and plant material:

The fresh aerial leaves of *Phyllanthus amarus* was collected from the local garden in Rajakulam village, Kanchipuram. In the month of October 2023.

Identification and authentication of plant material:

The collected specimens were botanically identified and authenticated by Dr.N.K.Sunil Kumar, Research Officer, and Department of Pharmacognosy. Siddha central Research institute, Chennai. 600 106. The sample was identified as Phyllanthus amarus belongs to the family Euphorbiaceae.

Plant profile:
Phyllanthus Amarus

VERNACULAR NAMES

English	Gulf leaf flower
Tamil	Kilannelli
Malayalam	Kilukanelli
Telugu	Nela usinka
Bengali	Bhuiamla
Hindi	Jamgli amli
Sanskrit	Bhumyamalaki
Assam	Amalakh

TAXONOMICAL CLASSIFICATION

Kingdom	Plantae
Division	Angiospermae
Class	Dicotyledonae
Order	Tub florae
Family	Euphorbiaceae
Genus	*Phyllanthus*
Species	*Amarus*

Extraction of plant material:
The plant leaves were collected and thoroughly washed with running distilled water into 2-3 times, to remove the dust sand and soil on the leaves were shade dried in 7-10 days [4]. at room temperature (25℃) to eliminate the excess amount of water. The dried plant leaves are grinded into the fine powder by using the electric grinder, and it further passed into the (sieve no:16) then stored into closed container for further use [9].

A (150 g) of herbal powder were macerated into the (300 ml) of 95% ethanol and enclosed by the aluminum foil sheet to avoid the contamination and left into the 3 days at room temperature [2: 6 w/v]. The solution was filtered into the Whatman filter paper then, the filtrate was placed in the rotator evaporator to remove the excess amount of water molecules, the extract was obtained at greenish semi- solid form, then extract was weighed 10g is obtained [5].

Extraction (Figure. 2)

Phytochemical screening:

s.no	Identification Test	Observation	**Plant extract (leaves) *Phyllanthus amarus***
1.	Alkaloids		
	A. Test extract + Wagner's reagent	Reddish brown ppt	Presence of alkaloids
	B. Test extract + tannic acid solution	Buff coloured ppt	Presence of alkaloids
2.	Carbohydrates		
	A. Aqueous extract + molish reagent shake and concentration H_2SO_4 from inside of the tube	Violet colour ring at junction of 2 layers	Presence of carbohydrates
	B. Aqueous extract + Fehling A and B reagents boil on water bath	Brick red ppt	Presence of carbohydrate
3.	Tannins		
	A. Test extract + 1% solution of gelatin containing 10% of sodium chloride	White ppt	Absence of tannins
	B. Test extract + lead acetate solution	White ppt	Absence of tannins
4.	Flavonoids		
	A. Test extract + sodium chloride	Intense yellow colour	Absence of flavonoids
	B.Test extract + lead acetate	Yellow colour ppt	Absence of flavonoids
5.	Amino acids		
	A. Aqueous extract + million reagent	White ppt	Presence of amino acids
	B. Aqueous extract + ninhydrin solution, boil	Purple colour	Presence of amino acids
6.	Terpenoids		
	A. Extract + filter paper	Not permanently stained	Presence of terpenoids
	B. Extract + alcohol	Soluble	Presence of terpenoids

Presence (+) Absence (-)

Formulation development:

Phyllanthus amarus (ointment) Ingredients

- Wool fat
- Hard paraffin
- Cetostearyl alcohol
- White soft paraffin

Formulation:

S.no	Ingredients	F1	F2	F3	F4
1	Phyllanthus amarus(extract)	0.10	0.20	0.30	0.40
2	Wool fat	2.5	2.5	2.5	2.5
3	Hard paraffin	2.5	2.5	2.2	2.3
4	Cetostearyl alcohol	2.4	2.3	2.5	2.3
5	White soft paraffin	42.5	42.5	42.5	42.5

Procedure:

1. Hard paraffin and cetostearyl alcohol taken in a china dish kept on water-bath melt at 70℃.
2. Wool fat and white soft paraffin are taken in a beaker and melt at 70℃.
3. This two mixture are added and stir until form an ointment base then, added the extract stirring to form the herbal ointment. The prepared herbal ointments were placed in jars, labeled, and kept in a room temperature[10].

Evaluation:

Colour and odour: Apply ointment and visually inspect it.

PH: Standard buffer solution was used for pH meter calibration. After weighing and dissolving about 0.5g of the ointment in 50ml of distilled water, the mixture was left for 2hours before the pH was assessed[13].

Stability study: The stability studies are carried out for the prepared ointment temperature of 37°cfor 2 months.

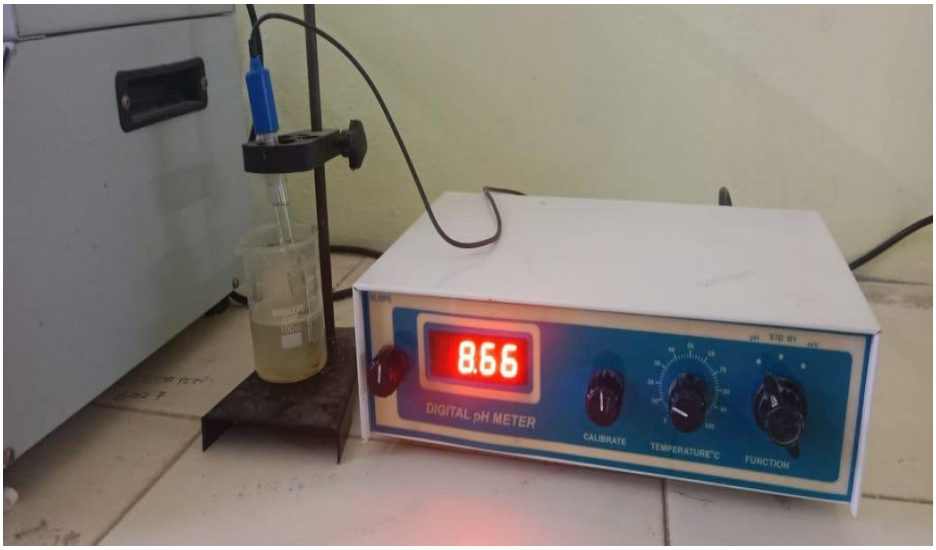
Diffusion study: An agar nutrient medium with any concentration was prepared in order to conduct the diffusion study. It was transferred into a china bowl. There was a centrally drilled hole filled with ointment. It was noticed how long it took for the ointment diffuse [12].

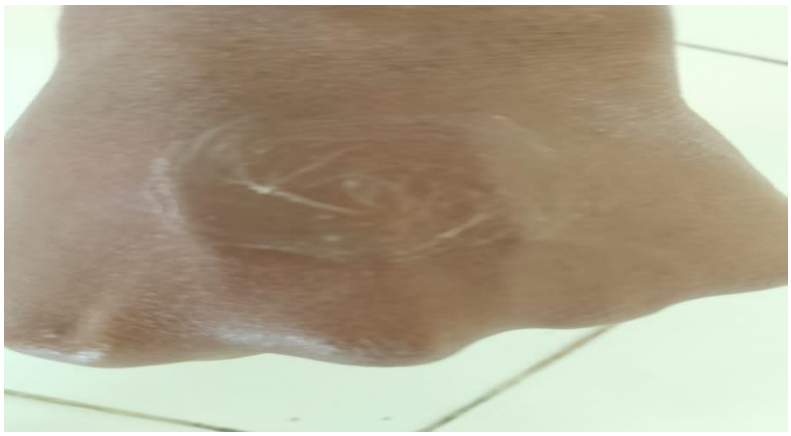
Loss on drying: Every 30minutes, 1g of ointment was added to the china dish and heated to 105ºC in the water bath until its weight stabilized.

Percentage loss on drying = (weight – molecular weight)/ weight × 100

Spreadability: The Spreadability was expressed in terms time in second taken by two slides to slip off ointment and placed in between the slides. Under the direction certain load. Lesser time taken for separation of two slides, result the better Spreadability was calculated by using the formula.

S = M.L/T





RESULT:

Phytochemical analysis:

s.no	Test name	Ethanol extract
1	Carbohydrate	+
2	protein	+
3	Reducing sugar	+
4	polyphenols	+
5	Flavonoids	+
6	Tannin	+
7	Cardiac glycoside	+
8	Anthocyanin	_
9	Quinones	_
10	Alkaloids	_

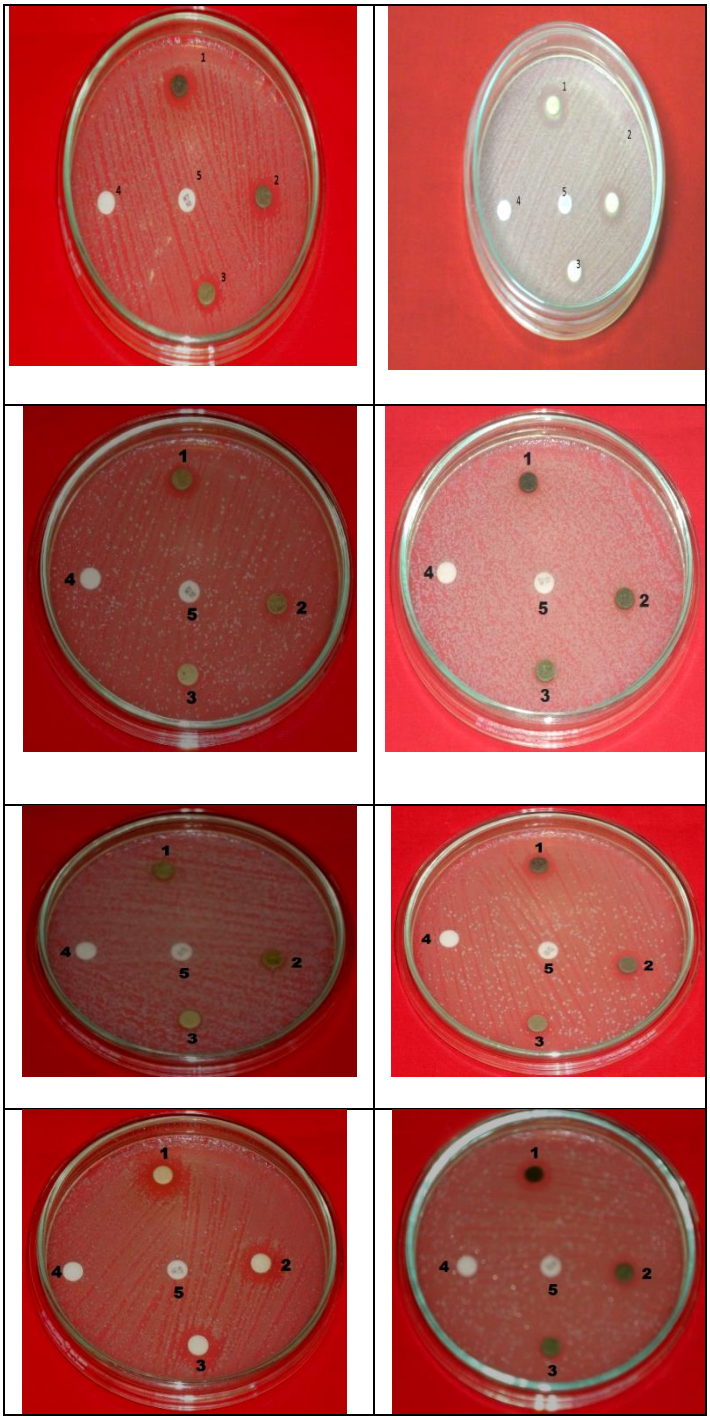
Evaluation:

s.no	Parameters	Result
1	Colour	Brownish – green
2	Ph	8.66
3	Loss of drying	23.7%W/V
4	Washability	Good
5	Spreadability	8
6	Solubility	Soluble in ethanol
7	Stability	Stable at 6 ph
8	Non- irritancy	No irritation occurred
9	Diffusion study	Within 35 mins
10	Odour	**Slight**

Table 1 Antifungal activity against ***Candida albicans and Candida Krusei***[11]

S. No	Compounds	**Mean zone of inhibition[a] (mm)[b] Concentration of the disc**					
		Candida albicans			***Candida Krusei***		
		250(µg/disc)	**100(µg/disc)**	**50(µg/disc)**	**250(µg/disc)**	**100(µg/disc)**	**50(µg/disc)**
1	Comp 1	13.6	10.8	8.8	15.5	13.8	8.2
2	Comp 2	9.5	6.8	5.6	13.5	11.2	6.3
3	Comp3	11.8	8.8	6.5	18.8	14.3	8.4
4	Comp4	16.5	10.8	10.1	22.8	11.3	9.3

Candida albicans ***Candida Krusei***



CONCLUSION:

In concluded that the present investigation comes out with the that *phyllanthus amarus* essential oil are required so that better, safe and cost effective drugs for treating S. aureus causing diseases. This study shows that Emblica Officinalis are antibacterial activity and have high potential as antibacterial agent. When the formulated as ointment for topical use and could therefore explain the successes claimed in the folk use of the plant in the treatment of common skin conditions. The potency of the herbal ointment against staphylococcus aureus could be hardness in the containment of the organism implicated as the commonest etiologic agent of boils, carbuncles,

infantile-impetigo and wound. The final product readily spread on skin surface, showed no irritant effect, diffused well and stable at different temperature. It also requires to research on phytochemical and pharmacological aspect. However, research going it would be easier to develop new formulation[2].

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