"PREPARATION AND EVALUATION OF ANTIMICROBIAL POLY-HERBAL FOOT BOMBS"

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ABSTRACT:
In the modern-day busy life with requirement of the different dress codes wearing shoes are must and caring for the most essential and most used part of body, the foot is less. As per the literature survey foot infections are very common to the populations and treatments for the same is costly and time consuming as the proper healing time will be less. It is also been observed that microbial infections due to various reasons like lack of hygiene, ill fitted shoe, contaminated due to exposure to the contaminated areas are common. In This Current research antimicrobial poly herbal effervescent foot soak was prepared and characterized to treat and manage the painful situation due to various foot infections. Plants like Ocimum sanctum, Camellia sinensis extracts, Sodium bi carbonate ,Potassium citrate ,Citric acid, Fluconazole, Corn starch ,MgSO4 ,Rose oil, were used to prepare the formulations in different ratio, where the best formulation was characterized by flowability characteristics, effervescent time , moisture content, short term stability studies, antimicrobial studies and interaction studies. FTIR shown no interaction between the drug and the excipients where effervescent time was found to be 12 seconds, In antibacterial study against *Staphylococcus* aureus the final formulation shown 14mm of zone of inhibition and 18 mm of zone of inhibition with drug fluconazole,In MIC, 74% zone of inhibition in 160 µg/ml.

KEYWORDS: Antimicrobial formulation , Antimicrobial foot bombs, Antifungal activity , poly herbal formulation , herbo- synthetic combination

INTRODUCTION:

Foot contaminations are agonizing circumstances that can cause from essential foot wounds incorporates various side effects, while causing more harm upon untreated. The feet contain 26 of bones and as per studies, an individual can walk gauges up to150,000 miles in the course of their life, generally equivalents to strolling the earth multiple times.
There are different foot and skin illnesses happen in our day today present-day life. Some of them are more and some are less infectious among us. Regardless of how clean you keep foots, they forever be in touch with growth or microorganisms. One can foster a foot sickness on the off chance that the individual has - low safe power, cut in foot skin, come roundabout contact with organisms. As pre the surveys it has been notice the Foot wounds (0.01-13.5%), foot contaminations 0.056.4%, aggregate foot infection (0.2-11.9%), where hazard factors included Fringe Blood vessel Sickness 0.01-36.0%, Fringe neuropathy 0.003-2.8%, and foot distortion were not revealed. Ulcer related foot wounds 4.6%, diabetes-related foot wounds 2.4%, and diabetes related foot contaminations 3.4%. This survey observed that foot illnesses are available in 1 in each 20 inpatients and principal risk calculate 1 of every 3 inpatients. [1,2]

Main causes for foot infections:

Below chart is representation of such causes: [3,4]

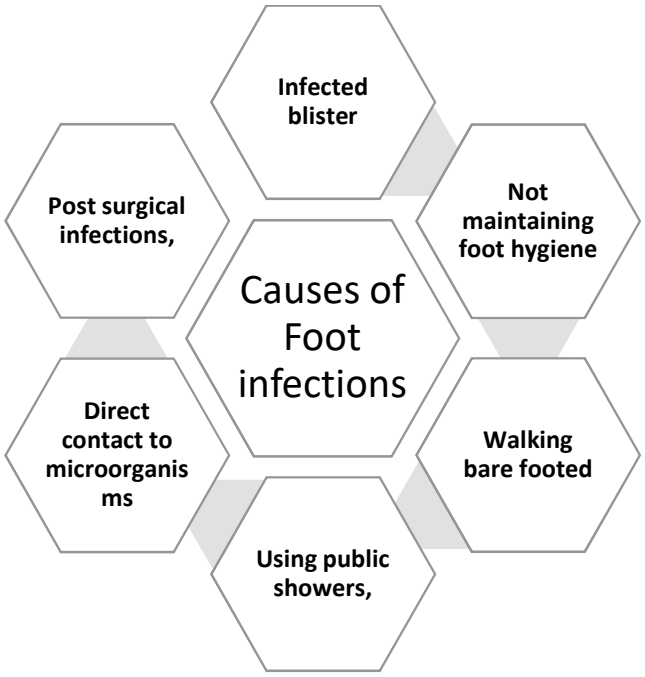


Chart:01

General Symptoms of Foot Infections:

Infected blister, Change in color, Warmth, Bad Smell, nail discoloration, Fever, Pus or fluid drainage, Pain, inflammations.

Table 1: Anti-microbial used for foot infections:

Orally administered Anti-biotics:	Amoxicillin Dicloxacillin Cephalexin.
IV route Anti-biotics:	Tazobactam Ciprofloxacin, Clindamycin, Piperacillin.
OTC Anti-fungal Agents:	Clotrimazole, Tolnaftate Terbinafine, Miconazole.

Table 2: Methods used for treating foot infections:

Excision:	This can be exceptionally helpful treatment for intense foot contaminations incorporates depleting of discharge and eliminates necrotic and tainted tissues.
Amputations:	Valuable when foot disease becomes gangrenous. It forestalls spreading of disease to different pieces of the body.

Vacuum-assisted closure:	Utilized in diabetic foot contaminations. Incorporates a gadget that diminishes pneumatic force around the injury, which mends the injury quicker. [5,6]

Importance to study about vitality of Foot infections:

Foot contaminations presently become truly challenging issues for doctor to treat as of biomechanical entanglements. At the point when a foot disease happens without diabetic condition, fringe vascular illness or other metabolic circumstances, it prompts harms of the delicate tissues might be of parasitic or microbes or both. In this way, it's vital to comprehend the basic reason for foot diseases consequently to treat or forestall the side effects as much as possible.[7]

Previously mentioned foot contaminations are extremely agonizing and undeniably challenging to recuperate with respect to day-to-day existence, we need to wear shoe consistently. Here in this examination, we had meant to plan natural non-poisonous foot bomb which will assist the patients with dealing with the side effect of foot contaminations and to recuperate quicker with next to no destructive synthetic compounds.

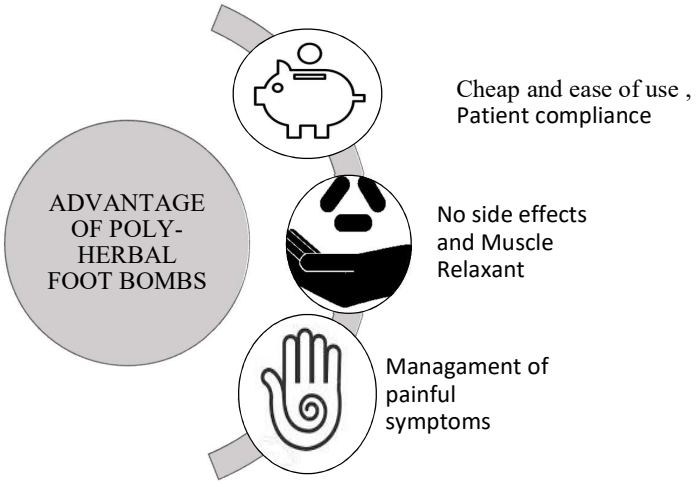


Chart: 02

Disease Profiling:

Athlete's Foot

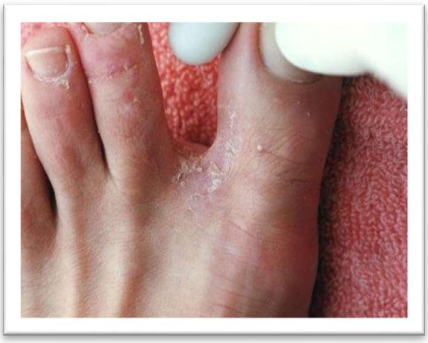


Fig:01

Name of the disease:
Athlete's Foot

Description and symptoms:
Can cause of Itchy skins of lower part of foot. This are caused by dermatophytes like- Trichophyton, Microspore.

Diagnosis:
Diagnosed By skin scraping or Potassium hydroxide test.

Treatment:
Anti-fungal creams / sprays, (OTC), Anti-fungal agents. [8,9,10]

Toenail fungus

Fig:02

Name of the disease:
Toenail fungus

Description and Symptoms:
Where Nails get separated from nail beds, getting decolourised to yellow and flaky state. Due to dermatophytes like-*Trichophyton, Microsporum*, and *Epidermophyton*.[6][7]

Diagnosis:
Diagnosed by Tissue culture of trimmed nail of patient/ subject can diagnosed to specific microbe, or KOH Test.

Treatments:
Oral Anti-fungal Treatment like – Terbinafine. [11,12]

Erythrasma:

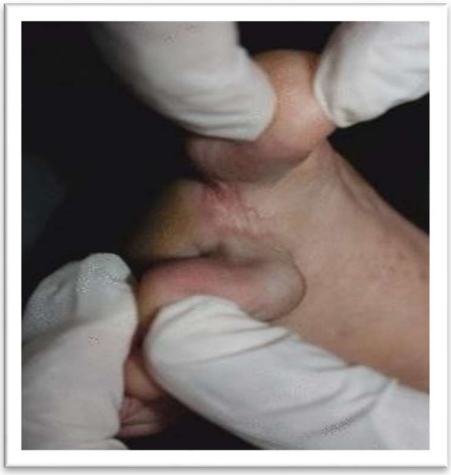


Fig:03

Name of the disease:

Erythrasma

Description and Symptoms:

Due to occurrence of a bacteria called *Corynebacteriumminutissimum*, generally caused by the diabetic patients, mainly to armpits, groin area, foots etc.[8]

Diagnosis:

Diagnosed by UV light test, called Wood's Lamp which gives a Coral-Pink glow to that bacterium.

Treatments:

Treated by Oral Antibiotics like- Azithromycin, Erythromycin. [13]

Foot Abscess:

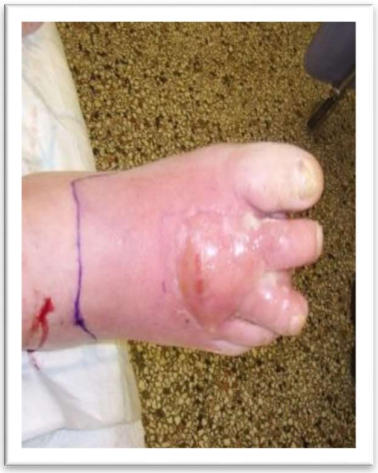


Fig:04

Name of the disease:

Foot Abscess

Description and Symptoms:

When there is a cut or wound then Bacteria can pass outer tissue layer, they cause formation of pus around it known as Abscess. Symptoms can be warmth, redness at that area, pain. Likely to get contaminated by *Arcanobacterium* pyrogens.

Diagnosis:

Diagnosed by Bacterial culture of infection area.

Treatments:

Treated by Draining the Abscess followed by oral antibiotics. [14,15]

Cellulitis:

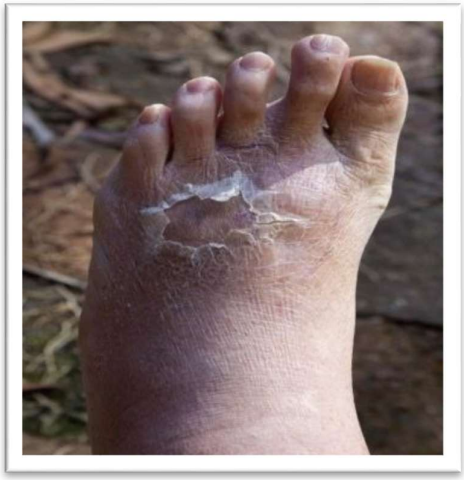


Fig:05

Name of the disease:
Cellulitis

Description and Symptoms:
A condition where a small cut gets contaminated with microbes and affects surrounding tissues, Symptoms are redness, pain, warmth followed by Body-ache, fever, body chills may cause by Streptococcus species, *Mucor* and *Aspergillus species* .

Diagnosis:
Diagnosed by Bacterial culture.

Treatments:
Treated by Antibiotic treatment. [16]

MATERIALS AND METHODS

Table 3: Material used

1.	**Plants Used**	*Ocimum sanctum* Leaves, *Camellia sinensis* Leaves
2.	**Chemical Used**	Fluconazole, Sodium Bi Carbonate, Potassium Citrate, Citric acid Corn Starch, Magnesium Sulphate, Rose Oil, Alcohol, agar-agar, sodium hydroxide, beef extracts
3.	**Apparatus Used**	Glass Beaker (250 ml, 100 ml, 50 ml), Measuring Cylinder, Petri dish

4.	**Equipment Used**	Hot air oven, incubator, autoclave, macerator, refrigerator, rota evaporator, thermometer, glass wares.

Pharmacogenetic profile:

Here is the detail drug morphology for research:

Ocimum sanctum:

Fig:06

Name:

Tulsi

Biological name:

Ocimum sanctum

Family:

Lamiaceae

Demography:

Ocimum sanctum is grown in semi-urban areas. It grows in almost every soil across world including India. *Ocimum sanctum* can grow from sea level to height of 2000m.*Ocimum sanctum* plant grows up to 3060cm with much branches on it. *Ocimum sanctum* having leaves with petiolate and ovate shape, up to 5cm length. Flower is purplish elongated racemes in close whorls.

Chemical Constitutes:

Ocimum sanctum contains various complex chemicals that vary according to their species. The leaf part of it contains – volatile oils like eugenol, eugenol, urologic acid, carvacrol, linalool, limatrol, caryophyllene etc, while the seed part contains fatty acids and sitosterol. *Ocimum sanctum* provides health endurance by containing saponins, flavonoids, triterpenoids, and tannins.

Uses: Anti-microbial activity:

Hydro-alcoholic extract of O. sanctum *L*. (60g/kg) provides antimicrobial activity over wide range of microbes like- *Klebsiella, E. coli, Proteus,* Staphylococcus *aureus* and *Candida albicans* when studied by agar diffusion method. [17,18,19]

Camellia sinensis:

Fig:07

Name:
Green tea
Biological Name:
Camellia sinensis
Family:
Theaceae
Demography:
Camellia sinensis is widely cultivated in –
China, India, Thailand, Argentina, Japan, Russia. These plants are medium sized growing up to height of 1.8m with 5-10cm length of leaves oval shaped and pointed at the tip. Leaves are generally finely dentate; Flowers are whitish, fragrant with 4-5 cm in diameter and having 5 petals
Chemical Constitute:
Camellia sinensis contains various complex constituents like: 15-20% proteins, **1-4% amino acids: -** glutamic acid, tryptophan, glycine, serine, aspartic acid, tyrosine, valine, leucine, threonine, arginine, and lysine. **Carbohydrates:-(5-7%)-** cellulose, pectins, glucose, fructose, and sucrose; **Minerals**: calcium, magnesium, chromium, manganese, iron, copper, zinc, molybdenum, selenium, sodium, phosphorus, cobalt, strontium, nickel, potassium, fluorine, and aluminium; Lipids, Sterols, pigments, Vitamins, Xanthine bases, and Volatile oils.

Uses:

Anti-microbial activity: *Camellia sinensis* shows anti-microbial activity over various selected Gram-positive and Gram-negative bacteria's because of presence of epigallocatechin gallate, which shows better activity over *Gram-positive* than *Gram-negative* like Staphylococcus aureus and Pseudomonas aeruginosa. *Camellia sinensis* also treats Mycotic by working against *Candida albicans* when given along with Anti-Mycotic. *Camellia sinensis* also treats Typhoid and Diarrhoea by working against *Helicobacter pylori*. Moreover, *Camellia sinensis* also treats influenza. [20,21,22,23,24]

Model Drug Profiling:

Fluconazole:

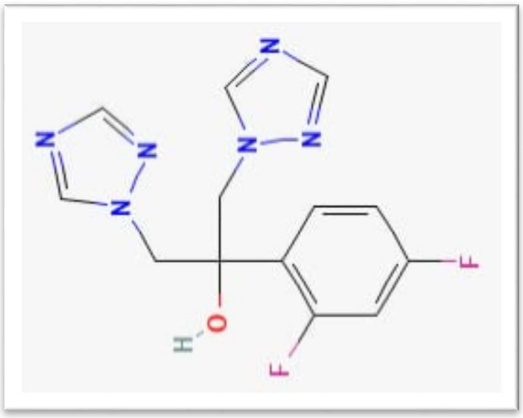


Fig:08

Molecular Formula:

[$C_{13}H_{12}F_2N_6O$]

Mechanism of Action:

Fluconazole communicates with 14-demethylase, a cytochrome P-450 catalyst answerable for catalysing the transformation of lanosterol to ergosterol. As ergosterol shapes a basic piece of the contagious cell layer, fluconazole restrains the union of ergosterol to increment cell porousness Microbiologically, fluconazole has action restricted to yeasts and some clinical movement against the endemic organisms, Histoplasma, Blastomyces, and Coccidioides.

Uses:

Fluconazole is an antifungal professionally prescribed medication endorsed by the U.S. Food and Medication Organization to: Treat mucocutaneous candidiasis, including *oesophageal candidiasis, oropharyngeal candidiasis* and *vulvovaginal candidiasis*. Forestall candidiasis in individuals going through bone marrow transplantation who are getting chemotherapy as well as radiation. Treat cryptococcal meningitis. [25,26]

Formulation of foot bombs:

Table 4: Formulation (5gm):

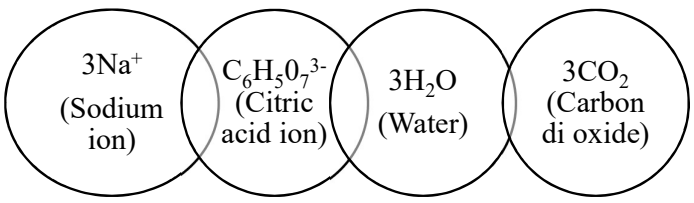
Sl no.	Ingredient	Quantity (per unit)	Use
1.	Sodium bi carbonate	2.050gm	Weak base
2.	Potassium citrate	0.520gm	Strong acid

3.	Citric acid	1.5 gm	Effervescent property
4.	Fluconazole	0.5gm	Model Drug
3.	Hydro-alcoholic extract of ***Camellia sinensis*** Leaves	Quantity Sufficient	Antimicrobial property
4.	Hydro-alcoholic extract of ***Ocimum sanctum*** Leaves	Quantity Sufficient	Anti-microbial property
5.	Corn starch	1.000gm	Binder
6.	$MgSO_4$	0.700gm	Muscle relaxant
7.	Rose oil	Quantity Sufficient	Fragrance

CALCULATION:

Here, we have calculated for 10 units of FOOT BOMBS:
Sodium bi carbonate: 10* 2.050gm = 20.50 gm
Potassium citrate: 10* 0.520 gm = 5.20 gm
Citric acid: 10* 1.5gm = 15gm
Corn Starch: 10*1.00gm = 10gm
MGSO4: 10* 0.700gm = 7gm
Rose oil: 1-2 drops.

Reactions:



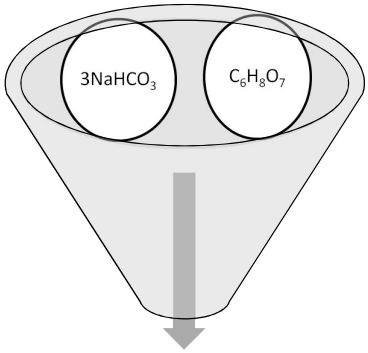


Chart: 03

Procedure for formulations of foot bombs:

- Preparing Hydroalcoholic extract of *Ocimum sanctum* and *Camellia sinensis* .
- Incorporation of extract to excipients
- Drying
- Adding model drug
- Blending and Moulding
- Evaluation

Preparation of extracts:

Hydro alcoholic extraction of *Ocimum sanctum:*

Ocimum sanctum leaves were collected from local vendors and identified by a certified Botanist.

↓

Leaves were cleaned properly with 2 to 3 times wash with tap water.

↓

Fig 09: *Ocimum sanctum* leaves

455 gm of leaves were subjected
to cold maceration with
30% alcohol (ethanol+ water).

↓

For continuous of 3 days with
shaking at intervals of some time,
and filtered,

↓

In the next step, a brownish
Solution was obtained
and placed in an air tight container until of use.

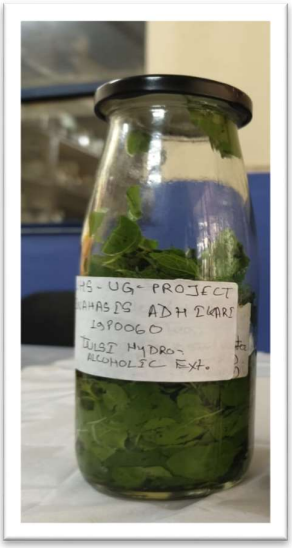


Fig10: Hydroalcoholic extract
of ***Ocimum sanctum***

Hydro alcoholic extraction of ***Camellia sinensis:***

Camellia sinensis was collected from local vendor
and identified by a certified botanist

↓

The *Camellia sinensis* was found as
dried powder form.

↓

Fig11: *Camellia sinensis* Leaves

After that, ethanol was added to required amount of powdered *Camellia sinensis* and followed by incubated at 28℃ for next 3 days.

↓

In the next step, the extract was filtered by Whatman Filter NO.1 and transferred to another 100ml glass beaker.

↓

The obtained brownish solution was kept in air-tight container until of use. [30]

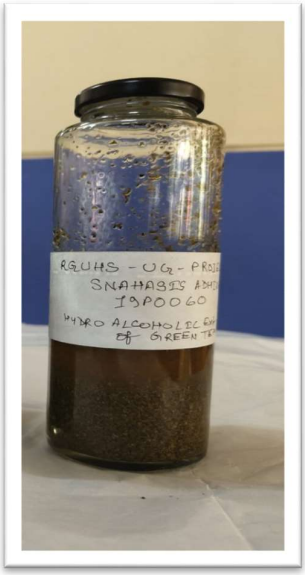


Fig12: **Hydroalcoholic extract of *Camellia sinensis* Leaves**

Incorporation of Excipients to Extract:

Accurately weighed 20.50gm of Sodium Bi carbonate was measured in a Petridis. *Ocimum sanctum* and *Camellia sinensis* extract were mixed and transferred into a glass beaker

Drying:

Measured 5 ml of combined extract to sodium bi carbonate and allowed to dry in Hoy air oven at 45ºC for 3 hours till the mixture becomes dried powder.

↓

Same procedure conducted till 15ml of combined mixture of extract was mixed and dried to sodium bi carbonate and form dried powder.

↓

After the combined mixture fully added to sodium bi carbonate, other excipients were added in the following order, Potassium citrate> Citric acid> Corn starch> Magnesium sulphate> Rose oil.

Addition of standard Drug:

Finally model drug, 0.5gm of FLUCONAZOLE was added to the powder and mixed.

Blending and Moulding:

In the next step, all the sample powder was blended properly in tumbling motion.

Overall Steps to Formulate Foot Bombs:

15ml of *Ocimum sanctum* leaves Extract and 15ml of *Camellia sinensis* Leaves Extract were transferred to a 100ml glass beaker to mix.

↓

20.50 gm of sodium bi carbonate was weighed and transferred to a Petridis.

↓

First, 5 ml of Mixture of extract was mixed with sodium-bi-carbonate and kept for drying for 90minutes in hot air over for drying.

↓

Same process repeated until 15ml of mixture extract was mixed dried with sodium bi carbonate properly.

↓

To that dried powder, weighed amount excipients were added as per the table: Properly mixed in tumbling motion.

↓

Model drug 500mg of fluconazole was added to that dried powder. Followed by blending again and mixed properly and moulded.

Fig13: Combined extract mixture

Fig 14: Sample Powder

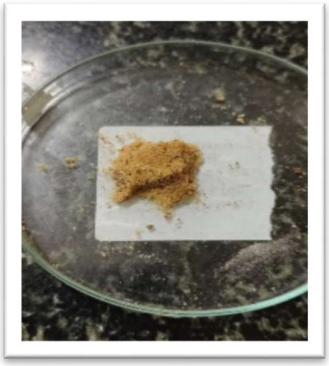


Fig15: Final Formulation

EVALUATION OF FOOT BOMB:

CHARACTERISTICS OF POWDER:

Bulk Density:

Still up in the air by estimating the volume of known mass of poly natural powder blend that has been gone through graduated chamber. The underlying volumes involved by the example was recorded. It is communicated as g/ml and determined by equation:

[Taken mass of powder / Bulk volume].

Tapped Density:

The Tapped not entirely settled by pouring the known mass of poly natural powder blend through a glass channel into a graduated chamber. The chamber was tapped from the level of 2 creeps until a steady volume got. It is communicated in g/ml and determined by equation:

[Taken Mass of powder / Tapped volume].

Angle of Repose:

The most extreme point which is shaped between the outer layer of a heap of concentrate and level surface is known as the point of rest. It was estimated by taking adequate measure of mixes which were permitted to go through a channel from a specific level of 2-4 cm to a level surface until it framed a store, which contacted the tip of the pipe. The level (h) and range[r] of the load were estimated. **Formula: Tan (ɵ) = (h/r)**

Carr's Index:

The compressibility file has been proposed as a backhanded proportion of mass thickness, size and shape, surface region, dampness content and cohesiveness of materials. Every one of these are firmly connected with foreseeing the powder stream characters. The compressibility not entirely settled by estimating both mass and tapped volume of the powder.

Formula: [(True density- Bulk density)/ Bulk density].

Determination of PH:

1. The PH meter was calibrated by standard buffer.
2. PH electrode was thoroughly rinsed with distilled water.
3. PH electrode was dipped in sample solution (1gm of formulation + 25ml of distilled water)
4. PH reading was displayed, and noted down when the instrument was stabilized.

Determination of Effervescent Time:

1. A clean 50ml of glass beaker was taken.
2. 25ml of distilled water was transferred to beaker.
3. 1gm of prepared sample solution was added and simultaneously timer was started.
4. The effervescent Tim was noted down.

Determination of Moisture Content:

1. The prepared formulation was weighed and transferred to a clean Petri dish (W1).
2. The Petri dish was kept in Hot air oven for 5 to 6 hours at 100C.
3. then the formulation weighed again (W2).
4. **Formula: [(W1-W2)/W1] *100**

2.1.1. Short-Term Stability Studies:

A detailed analysis of change in PH, Effervescent Time and Moisture Content was checked for 15 days at 5days interval.

RESULTS AND DISCUSSION:

Table 5: Observations of Characteristics of Sample Powder:

Study name	Results	Remarks
Bulk Density	0.718 g/ml	
Tapped Density	0.894 g/ml	
Angle of Repose	20.31	Flowability- Excellent
Carr's Index	21.09	Flowability-Fair
Hausner's Ratio	1.24	Flowability-Fair

Table 5.1: Observation on Short-Term Stability Studies:

Test	1st Interval	2nd Interval	3rd Interval	Remark
PH	7.1	6.9	6.9	Stable
Effervescent Time	12sec /gm	10sec /gm	8sec /gm	Slight Decrease in Effervescent Time
Moisture Content	[(16.48-14.33)/16.48] *100 = 13.046%	14.28%	15.14%	Slight Increase in Moisture Content

4.0 FTIR studies:

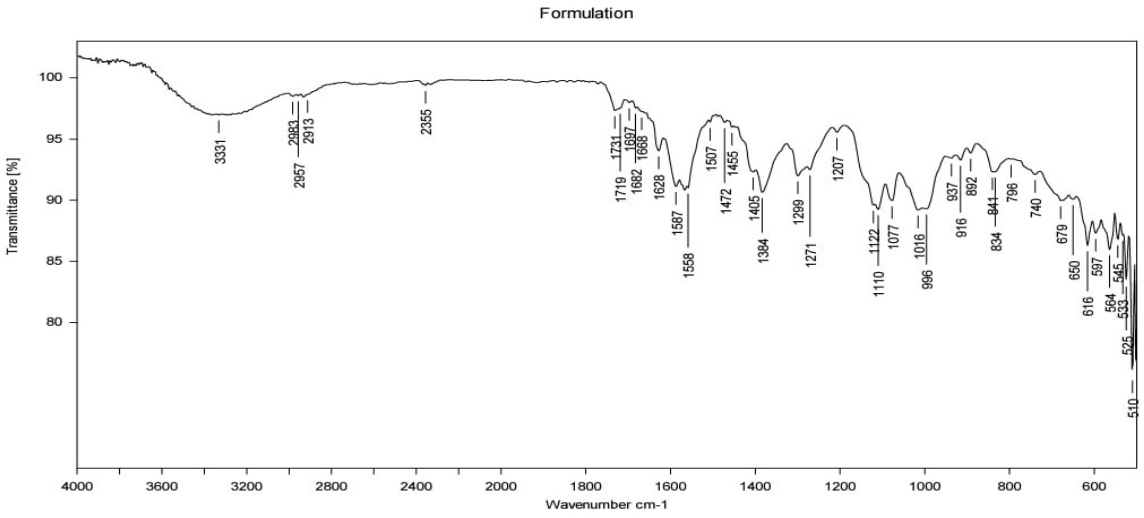
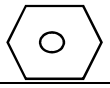


Table 6: FTIR Interpretation

Bond	Wavenumber(cm-1)
O-H Stretching	3333-3267
C=N	1650-1550
C-F stretching	1400 – 1100
N-H Stretching	1587

	1600
COO stretching	1558
C-H Bending	2355
C=C Bending	1719 – 1731
C=O Stretching	1720-1702
N=N Stretching	1384
C-H Stretching	1385-1380
C=C Bending(monosubstituted)	995-980
C-H Bending (1, 3 disubstituted)	880±20

ANTIMICROBIAL STUDY ZONE OF INHIBITION:

Materials & Methods:

Sample 1: *Ocimum sanctum* Leaves Extract
Sample 2: *Camellia sinensis* Leaves Extract
Sample 3: *Ocimum sanctum* Leaves + *Camellia sinensis* Leaves Combined Extract
Sample 4: *Ocimum sanctum* leaves extract + *Camellia sinensis* leaves extract + Fluconazole

Table 7: Requirements

Sl .No	Particulars	Source	Catalogue No
1	Agar Base	Himedia	90935
2	Petri plates	Genaxy	GEN-PTD-90
3	96 well plate	Thermo Scientific	167425
4	L-spreader	Himedia	PW1085-1x20N0
5	1000µl tips	Genaxy	GENUT 1000C
6	200µl tips	Genaxy	GENUT 200C
7	Micro centrifuge tubes	X pet	MCT-1.7-B

Test organisms:
Staphylococcus aureus

Test compound as standard:
Tetracycline

Inoculum:
Staphylococcus aureus cell suspension were prepared and grown on media and cultures were incubated for 24hrs at 37°C. The cell suspensions of all the cultures were adjusted to 1-2x 10^6cells/ml.

Test compound:
Sample: (concentration will be change as per requirements)

S-Standard:
Tetracycline 25 µg/ml

Procedure:

Determination of Antimicrobial activity:
Staphylococcus aureus were inoculated on media (90 mm)
Test compounds: Sample (25µl), Standard Tetracycline (25µl) *Staphylococcus aureus* were added to the 5mm well on agar plates. The treated plates with *Staphylococcus aureus* were incubated in

aerobic chamber at 37°C for 24hrs. The treated plates were observed for zone of inhibition around the wells

Results:

Table 8: Inhibitory activity of test compounds against test organisms

Test Organisms	Test Compounds	Conc. per well	Zone of inhibition (mm)
Staphylococcus aureus	Control	-	-
	Tetracycline (Standard)	25 µg/ml	22
	Sample 1 (25µl)	250 µg/ml	10
	Sample 2 (25µl)	200 µg/ml	13
	Sample 3 (25µl)	200 µg/ml	14
	Sample 4 (25µl)	200µg/ml	18

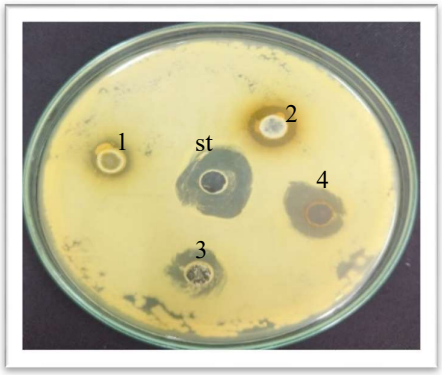


Figure 16: Inhibitory activity of test sample against Staphylococcus *aureus*
S-Standard (Tetracycline).1 – sample 1 ,2 -sample 2, 3-sample 3 ,4-sample 4

MIC TEST:

Test organisms:

Staphylococcus aureus

Sample preparation:

10mm stock solutions will be prepared by using DMSO. From that stock solution dilutions will be made up to0.161µM for the initial screening

Standardized Microbial suspension:

The bacterial inoculum was prepared in accordance with the CSLI recommendation where the OD_{600} value was adjusted to the equivalent of McFarland equivalence turbidity of 0.5 (10^8 CFU /mL), which was confirmed from a calibration curve for each microorganism.

Inoculum: Cell suspension prepared from bacterial cultures grown on LB broth and cells were adjusted to 1-2 x 10^8cells/ml.

Standard preparation: Stocks was twofold diluted in broth to give 5, 2.5, 1.25, 0.625, 0.312, 0.156 and 0.078µM.

Drug concentrations: drug concentration prepared:

Tetracycline (8µg/ml): 30, 15, 7.5, 3.75 and 1.8 µg/ml in LB broth.

Test compounds (Sample):

160,80,40,20 and 10 in twofold dilution broth.

Control: LB broth was inoculated with respective culture and without test compounds

Procedure:

CLSI broth micro dilution method.

i. Mix 90µl test samples / standard of different test concentration with 10µl inoculum (1-2×10^8) in 96 well plates.

ii. Control: Mix 90µl LB broth without drug with 10µl inoculum in 96 well plates.

iii. After incubation for 24 h at 37 °C, resazurin (0.015 %) was added to all wells (30 µl per well), and further incubated for 2–4 h for the observation of colour change. On completion of the incubation, columns with no colour change (blue Resazurin colour remained unchanged) were scored as above the MIC value.

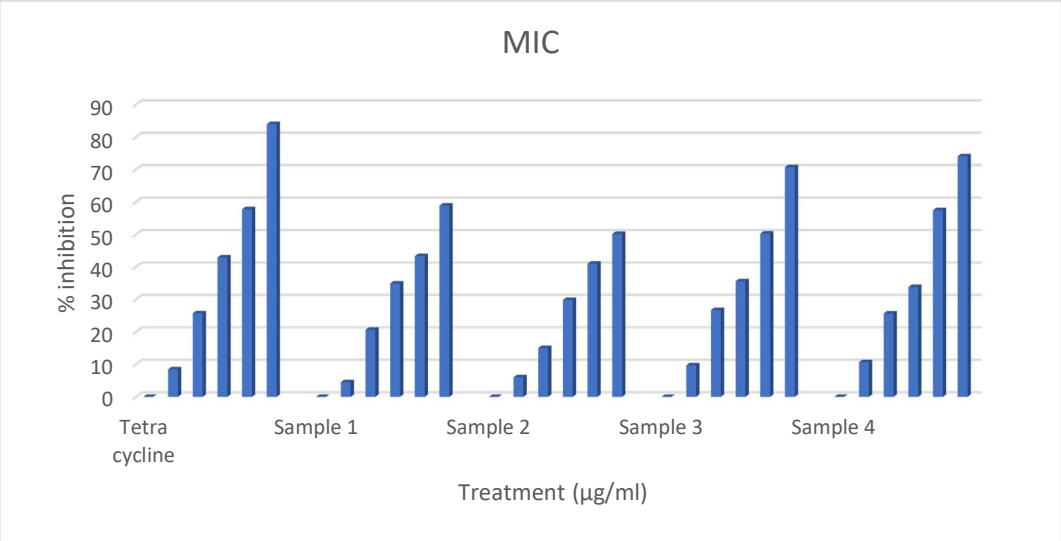
iv. Metabolism of Resazurin by active bacterial cells leads to reduction of Resazurin (Purple-blue) to resorufin (*pink-colourless*) Pink colour.

Result:

Table 9: **Minimum inhibitory activity of tested compound against test organisms**

	Concentration (µg/ml)	OD@517	%Inhibition
Tetracycline	**Control**	0.732	0
	1.8	0.67	8.47
	3.75	0.544	25.68
	7.5	0.42	42.88
	15	0.31	57.78
	30	0.117	84.02
Sample 1	**Control**	0.732	0.00
	10	0.699	4.51
	20	0.581	20.63
	40	0.477	34.84
	80	0.415	43.31
	160	0.301	58.88
Sample 2	**Control**	0.782	0.00
	10	0.738	6.01
	20	0.672	15.03
	40	0.564	29.78
	80	0.482	40.98
	160	0.415	50.14
Sample 3	**Control**	0.742	0.00
	10	0.67	9.70
	20	0.544	26.68
	40	0.478	35.56
	80	0.369	50.26
	160	0.217	70.75

Sample 4	**Control**	0.722	0.00
	10	0.645	10.66
	20	0.537	25.62
	40	0.478	33.78
	80	0.307	57.48
	160	0.187	74.10



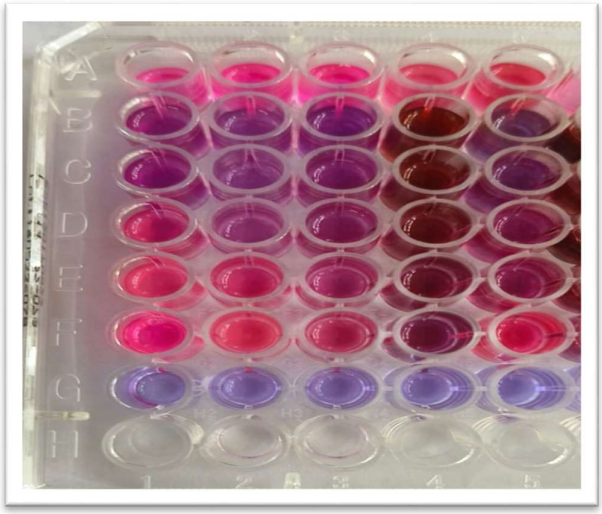


Fig17: MIC TEST

ANTIFUNGAL ACTIVITY BY WELL DIFFUSION METHOD:

Table 10: Materials & Methods

Sl.No	Particulars	Source	Catalogue No
1	PDA Agar Base	Himedia	MCD096
2	Petri plates	Genaxy	GEN-PTD-90
3	96 well plate	Thermo Scientific	167425
4	L-spreader	Himedia	PW1085-1x20N0
5	1000µl tips	Genaxy	GENUT 1000C
6	200µl tips	Genaxy	GENUT 200C
7	Micro centrifuge tubes	X pet	MCT-1.7-B

Test organisms:

Candida albicans

Test compound as standard:

API

Inoculum:

Candida albicans cell suspension were prepared and grown on media and cultures were incubated for 24hrs at 24-30°C. The cell suspensions of all the cultures were adjusted to 1-2x 10^6spores/ml.

Test compound:

Sample – 500µg/ml
Standard: API100 µg/ml

Procedure:

Determination of Antifungal activity:

Candida albicans were inoculated on media (90 mm)

Test compounds:

Sample (25µl), Standard API (25µl) *Tinea corporis* were added to the 5mm well on agar plates.

The treated plates with *Candida albicans* were incubated in aerobic chamber at 24-30°C for 24hrs.

The treated plates were observed for zone of inhibition around the wells.

Results:

Table10: Inhibitory activity of test compounds against test organisms

Test Organisms	Test Compounds	Conc. per well	Zone of inhibition (mm)
Candida albicans			
	API (Standard)	25 µg/ml	20
	Sample (25µl)	250 µg/ml	11

Figure 18: Inhibitory activity of test sample against *Candida albicans* S-Standard (API). A – sample

CONCLUSION:

Upon conducting above Anti-microbial, MIC and Anti-fungal Tests, it can be concluded that both the plants showing synergistic action in inhibiting microbial growth. In current study, we can presume that there are different foot diseases made by different reasons beginning from microbial defilement ways of life and keep up with cleanliness. It is additionally seen that those foot diseases are hard to treat as in our day-to-day existence we should wear shoes and we should walk. These foot diseases are additionally hard to treat as numerous causative specialists presently become impervious to many customarily utilized enemy of microbial. In various examinations it has been found different plant having cell reinforcement and substance constituents having against microbial exercises. Thus, in the wake of doing this Exploration a drive can be taken to form a poly-natural detailing as fix or effective or as foot plunge definition to deal with the difficult side effects of those diseases.

FUTURE SCOPE:

In Further Research activity of the formulation against more pathogens, bacteria and fungi can be explored, activity against the resistant pathogens can be explored. Antioxidant activity and animal studies can be performed

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