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PRELIMINARY PHYTOCHEMICAL INVESTIGATION AND ANTIMICROBIAL ACTIVITY OF HERBAL OINTMENT

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ABSTRACT

The aim of the study was assess the antimicrobial effect of some medicinal plants and its formulation against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Medicinal plants are the important source of potentially useful structures for the development of novel chemotherapeutic agents. Historically, plants have provided a source of the development for novel drugs and plant derived drugs which have made large contributions to human health and wellbeing. The antibacterial activities of selected plants extracts were evaluated using the disk diffusion method as well as cup plate method. Zones of inhibition were observed in disc diffusion for antimicrobial investigation against Gram-positive and Gram-negative pathogenic bacteria. The Polyherbal formulation showed average zone of inhibition ranged from 6-8 mm.

KEYWORD: Phytochemical investigation, medicinal plant, antimicrobial activity, Polyherbal formulations, Ointment.

INTRODUCTION

Herbs and products containing herb(s) have been in trade and commerce and are currently used for a variety of purposes. These therapeutic formulations may also be referred to as drugs or botanicals, or when taken orally to provide health benefits, they may be called

dietary supplements or even food ingredients in some cases. Herbal medicines are plant derived materials and preparations with therapeutic or other human health benefits, which contain either raw or processed ingredients from one or more plants, inorganic materials or animal origin. Herbal medicine preparations are developed and created drugs by the modern pharmaceutical industry. Nowadays, they are manufactured and sold most widely on the pharmaceutical market for curing diseases and promoting public health in India. Of the 2, 50,000 higher plant species on earth, more than 80,000 are medicinal. India is one of the world's 12 biodiversity center with the presence of over 45000 different plant species. India's diversity is unmatched due to the presence of 16 different agriculture climatic zones, 10 vegetation zones, 25 biotic provinces and 426 biomes (habitats of specific species). Of these, about 15000-20000 plants have good medicinal value. However, only 7000-7500 species are used for their medicinal values by traditional communities. In India, drugs of herbal origin have been used in traditional systems of medicines such as Unani and Ayurveda.[1] In that formulation antimicrobial properties drug used as *Curcuma caesia,*[2] *Bacopa monnieri,*[3] *Tinospora cordifolia,*[4] *Moringa Oleifera,*[5] *Caesalpinia bonducella*[6] *Citrullus lanatus,*[7] *Murraya koenigii,*[8] *Evolvulus alsinoides,*[9] *Gymnema sylvestre,*[10] Withania Coagulans[11] that is polyherbal drugs shows antimicrobial activity to used. Semisolid dosage forms include ointments and creams. 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 or be absorbed through the skin for systemic action. Many ointments are made from petroleum jelly. Like many other pharmaceutical preparations, they frequently contain preservatives and may also contain aromatic substances and dyes to enhance patient acceptance. Although there is generally no agreed-upon pharmaceutical definition for creams, they are very much like ointments in their use. Their composition is somewhat like that of ointments except that creams often have water-in-oil emulsions as the base of the formulation. When applied to the skin, creams feel soft and supple and spread easily. Method of preparation of ointment that is Firstly Trituration, and lastly Fusion. In trituration method finely subdivided insoluble medicaments are evenly distributed by grinding with a small amount of the base followed by dilution with gradually increasing amounts of the base. And In Fusion method the ingredients are melted together in descending order of their melting points and stirred to ensure homogeneity.[12]

MATERIALS AND METHODS

Antibiotics activity of these compounds has been assayed against two different of bacteria (one gram-positive and one gram-negative) by agar diffusion method. Generally, the antibiotics activity of a compound is expressed in terms of its ability to inhibit the growth of bacteria in nutrient broth or agar. The bacterial inhibition can be measured by two methods First one Serial dilution method and second one diffusion method. The serial dilution method is very much useful for the determination of the antibiotics activity. But it is not much useful for the qualitative detection tests and also for the evaluation of large number of compounds.[13] In this method technique used a simple device is used to remove the disc of agar from the medium. This instrument' consists of a thin-walled stainless steel cylindrical chamber measuring 2.5 cm. in length and having a diameter of 1.5 cm.; the cutting edge is bevelled on the inside. A capillary metal tube, about 10 cm. long, is attached to the bottom of the cylindrical chamber. The chamber is sterilized by dipping in alcohol and flaming. Placing the open end of the chamber on the surface of a poured agar plate, the disc is cut easily with slight pressure.[14] Agar cup plate method is one of the official methods in IP, where the test samples diffuse from the cup through an agar layer in a Petri dish or plate to such an extent that the growth of added microorganisms is restricted entirely to a circular area or zone around the cavity containing the solution of an antibiotic substance. The antimicrobial activity is expressed as zone diameter in millimeters, which is measured by a scale. Gram-positive organisms used in the study were *Staphylococcus aureus*, Gram-negative organism used was *Pseudomonas aeruginosa* and *Escherichia coli.* Material used as Nutrient agar (Hi media) preparation of nutrient agar volumes of peptone (0.6%), yeast extract (0.15%) and di-potassium dihydrogen phosphate (0.36%) were dissolved in distilled water and the pH was adjusted to 7.2. This solution was sterilized by autoclaving at 15 psi for 20 minutes. Preparation of peptone water Definite volumes of peptones (0.6%), yeast extract (0.3%) and beef extract (0.13%) were dissolved in distilled water and the pH adjusted to 7.2 and sterilized by autoclaving. Agar diffusion assay (Disc diffusion method, Disc size 6 mm)[15]

Preparation of formulation

- **Preparation and Evaluation of ointment**

Two topical ointment bases of varying degrees of aqueous/anhydrous character namely: simple ointment BP and emulsifying ointment BP were prepared by fusion method (Table 1). The constituents of the base were placed together in a melting pan and allowed to melt at 70°C. After melting, the ingredients were stirred gently at 70°C for 5 minutes and then cooled

with continuous stirring. Incorporation of 1g of the extract into the various bases was achieved by triturating in a ceramic mortar with a pestle to obtain 10 g of herbal ointments. The prepared herbal ointments were put in ointment jars, labelled and were stored at 25^0C. The two formulated ointments and the standard, Nuforce-GM cream were evaluated for the following parameters: appearance, odour, texture, spread ability, homogeneity and irritant effect using the method employed by Alalor *et al.*

In vitro antimicrobial efficacy of formulated ointments was carried out using the cup-plate method.[16]

- **Preparation of test solution**

To small amount of extract equal volume of 2M hydrochloric acid is added and heated the test tube for 30-40 min at 100^OC, allowed to cool, filtered and extracted with ethyl acetate. The ethyl acetate extract was concentrated to dryness, followed the test for flavonoids to ethyl acetate fraction by dissolving the residue with ethyl acetate.

a) Shinoda test: Test solution with few fragments of magnesium ribbon and conc. hydrochloric acid shows pink to magenta red colour.

b) Zn/Hcl reducing test: Test solution with zinc dust and few drops of hydrochloric acid shows magenta red colour.

- **Test for Phenolics/Tannins**

The extract is dissolved in 90% alcohol.

a) *Ferric chloridetest* Test solution treated with few drops of ferric chloride solution gave dark colour.

b) *Gelatin test* solution treated with gelatin gives white precipitate.

- **Tests for proteins**

The extract is dissolved in water.

a) Millon's test: Test solution is treated with reagent and heated on a water-bath, protein is stained red on warming.

Biuret test: Testsolution treated with 40% sodium hydroxide and dilute copper sulphate solution have given blue colour.

Table 1: Preliminary phytochemical investigation of aqueous extracts of selected medicinal plant.

Name of Phytoconstituents	Curcuma caesia	Evolvulus Alsinoides	Citrullus lanata	Withania coaglancce	Tinospora cordifolia	Gymnema sylvestra	Caesalpenia bonduc	Bacopam onnieri	Moringao leifera	Murraya koenigii
	Aqueous	Aqeous	Aqueous	Aqueous	Aqueous	Aqueous	Aqueous	Aqueos	Aqueous	Aqueos
Alkaloides	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve
Glycosides	+ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve
Saponins	+ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve
Steroides	-ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve
Terpenoides	-ve	-ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve
Flavonides	-ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve
Tannins	-ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve
Carbohydrate	+ve	+e	-ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve
Phenolic	+ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve
Protein	+ve	--	-ve	--	-ve	--	-ve	+ve	+ve	+ve

Table 2: Formulation A (for preparation 10g.)

Ingredient	Quantity given	Quantity taken
Extract	10g.	1g.
Cetostearyl Alcohol	4.5ml	0.45ml
Hard Paraffin	4.5g.	0.45g.
White Soft Paraffin	76.5g.	7.65g.
Wool Fat	4.5g.	0.45g.

Table 3: Formulation B (for preparation 5g.)

Ingredient	Quantity given	Quantity taken
Extract	10g.	500mg
Liquid Paraffin	18ml	0.9ml
Emulsifying Wax	27g.	1.35g
White Soft Paraffin	45g.	2.25g.

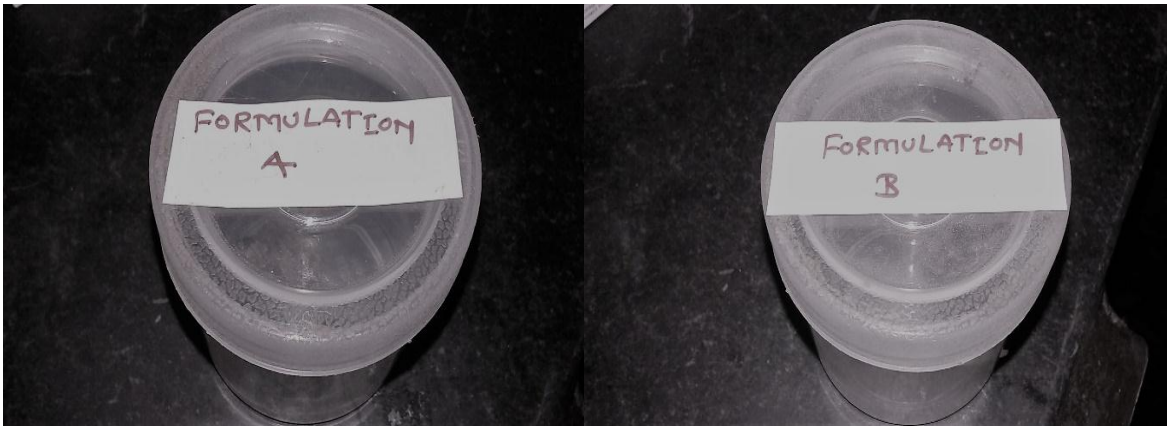
Table 4: Culture method.

Microorganism	Strain Name	Strain Reference
Gram Positive Bacteria	Stophylococus aureus	NCIM 2079
Gram Negative Bacteria	Pseudomonas aernginosa	NCIM 2036
	Escherichia Coli	NCIM 2109
NCIM : National Collection of Industrial Microorganisms, National Chemical Laboratory (NCL), Pune 411008 [India]		

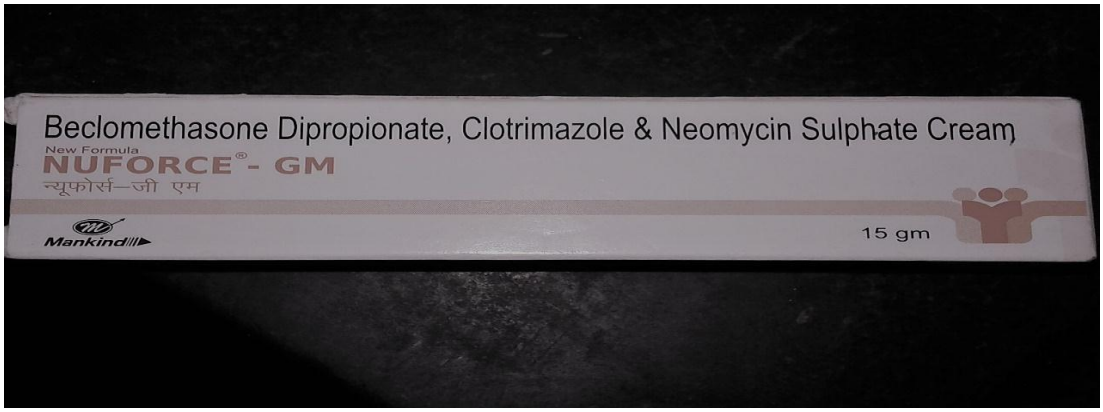
Media used

Microbiological media used for bacteria (*B. subtilis, Staphylococcus aureus and Escherichia coli)* is Nutrient agar (Himedia)

E. Composition (gL-1)**:** Sodium chloride, 5.0; Beef extract 10.0; Peptone 10.0 (pH 7.2)



Formulation- A **Formulation- B**

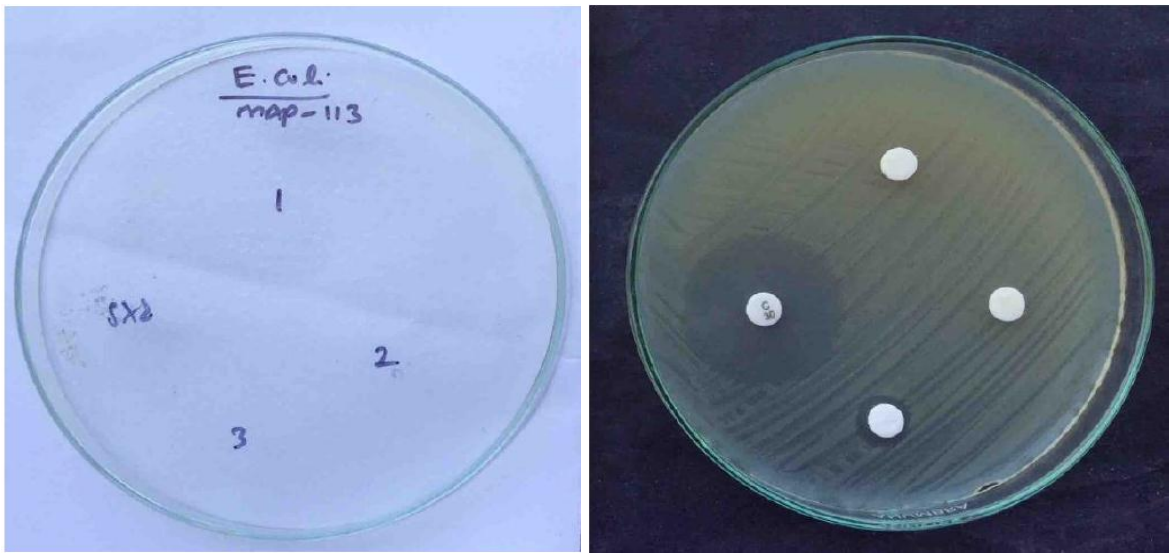


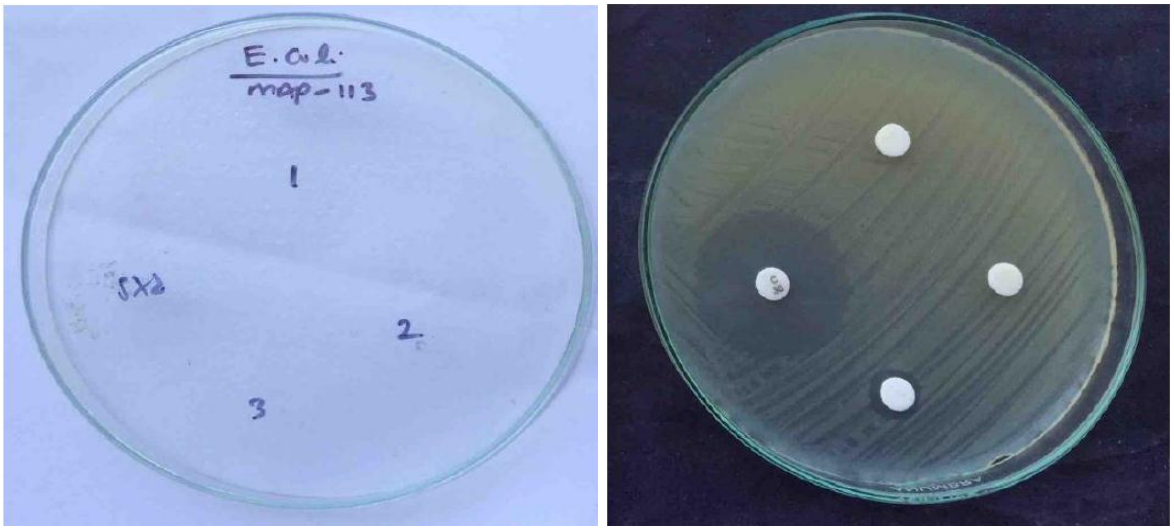
Std. Formulation

Table 5: Result and Discussion.

Sr. no.	Sample code	S. aureus	E. coli	P. aeruginosa
1.	A	7.08	7.30	6.50
2.	B	7.38	7.34	6.59
3.	Std.	12.51	9.49	8.63
4.	Chloramphenicol	28.79	27.82	15.04

The antibiotics activity of these compounds has been assayed against two different of bacteria (one gram-positive and one gram-negative) by agar diffusion method. Generally, the antibiotics activity of a compound is expressed in terms of its ability to inhibit the growth of bacteria in nutrient broth or agar. The bacterial inhibition can be measured by two methods, that show as the (table-5) the sample A and B against to standerd formulation to S. agureus - 7.38+_, E.coli-7.30+_, P. aeruginosa- 6.50+_ as per standerb formulation compare on sample A and B.

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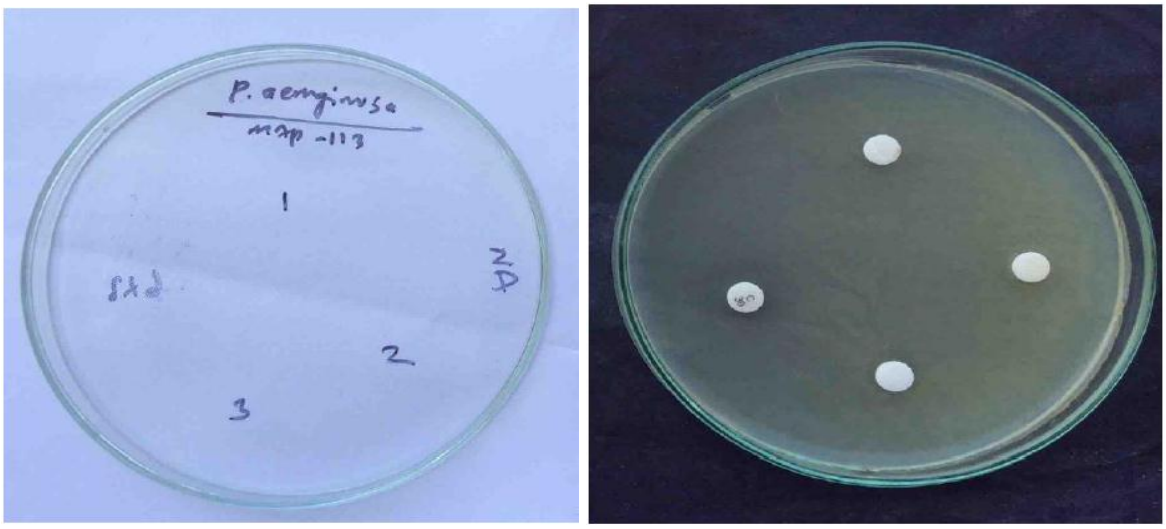
P. aeruginosa
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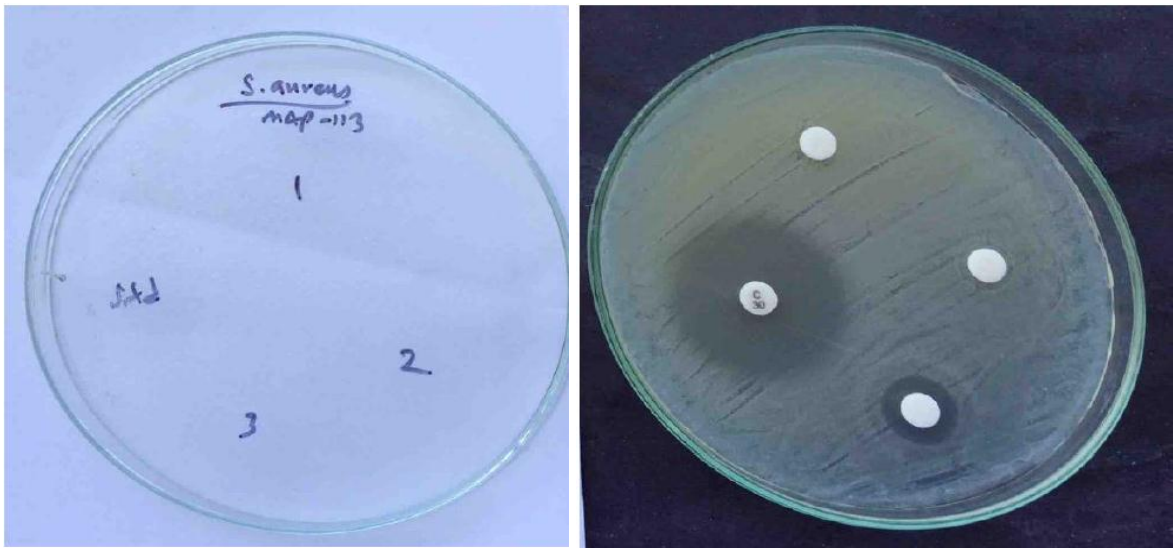
S. aureus
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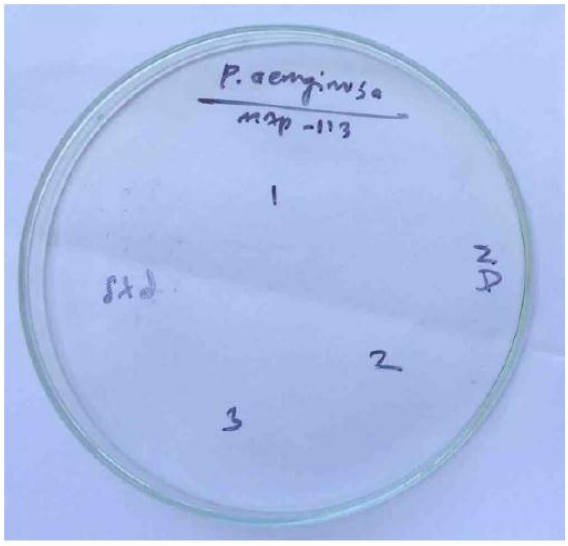

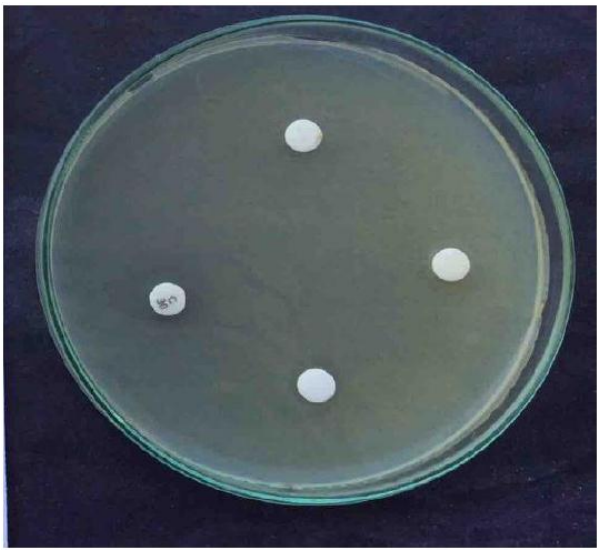

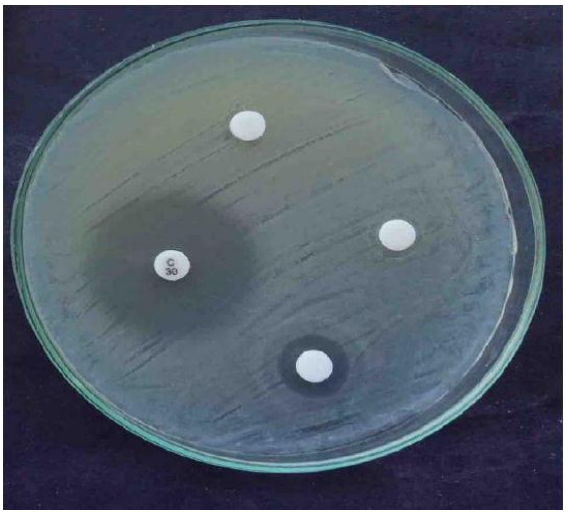

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