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A COMPARATIVE STUDY FOR ANTIMICROBIAL ACTIVITY WITH AMOXYCILLIN OF PREPARED POLYHERBAL FORMULATION

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Abstract: Herbal medicines are used in conventional medicine as well as in special medicine accomplished in the developed world. There were several herbal medicines are used for skin and skin related disorders. Thus, the main aim of the present work was to formulation & screening of antimicrobial activity of three herbal plants against human pathogenic bacteria like *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* & was determined by invitro agar diffusion method. The plants like *Erythrina indica* leaf, *Calatropsis procera* root & *Ficus religiosa* bark are selected and were evaluated for its Phytochemical constituents, physicochemical property & antimicrobial activity. The extract was prepared by maceration extraction process by using aqueous solvent, Chloroform solvent & Methanol solvent & also by Continuous hot extraction-soxhletation process by using Methanol solvent. The extracts were formulated indifferent concentration of 2%, 4% & 5% by using same solvent & compared with the standard antibiotic like Amoxicillin (10µg/ml). Agar well diffusion method was used and the extracts showed extensive zones of inhibition beside the tested isolates. Better antimicrobial activities were obtained with extract which gave better minimum inhibitory concentration (MIC) for the selected. In this study it has been showed that the methanol extracts prepared by hot extraction process (soxhletation) had wider range of antimicrobial activity on used organisms than the aqueous, chloroform & methanol extract by maceration extraction process. Which indicates that the methanol extracts by soxhletation of all selected plants may contain more active components. This study supports, the traditional medicines (herbal extracts) to cure many diseases like intestinal tract, diarrhea, ear infections, fever, throat and wound.

Keywords: Antimicrobial activity, *Erythrina indica* leaf, *Calatropsis procera* root & *Ficus religiosa* bark, Agar well diffusion method, Human pathogen.



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INTRODUCTION

When two or more plants are used for preparation of formulation, they are known as polyherbal formulations. Herbal medicine is making remarkable comeback and increasing number of patients visiting stand-by medicine clinics. Side effects of synthetic drug are shocking and recent time it has seen danger because of drug interactions ^[1]. Plant based antimicrobials stand for a huge intact genetic mechanisms of action of resistance and to continue studies source for medicines and additional investigation of plant to develop new drugs, either synthetic or natural. Antimicrobial of plant critical aim are to offer suitable and professional origin have huge remedial potential ^[2]. Now a day about 80% of the world's people in Asian, Latin American, African and Middle Eastern countries is using plants as traditional health remedies due to fewer side effects ^[3, 4]. Human antimicrobial infections mainly those involving microorganisms i.e fungi, viruses, bacteria, etc. they cause severe infections in tropical and subtropical countries of the world. In current years, numerous drug resistances in human pathogenic microorganisms has been developed due to use of marketable antimicrobial drugs frequently used in against microorganisms, as a result, plants are one of the treatment of such diseases ^[5, 6]. Antibiotics are one of the greater significant part beneficial discoveries of the 20th century that had support against stern bacterial infections but, only one third of the infectious diseases known have been treated from these synthetic drugs. This is because of the appearance of different pathogens that is absent from provision the importance of years of general casual use, nonstop and mistreatment of antibiotics ^[7]. The microorganism & bacteria have the genetic capacity to pass on and obtain resistance to drugs, which are utilized as therapeutic agents ^[8]. It is very dangerous to human being to obtain resistance in future so to overcome this reduces antibiotics & develop a new formulation from synthetic or herbal. Until a natural formulation have been approved as new antibacterial drugs there is an urgent need to identify novel substances active towards highly resistant pathogens ^[9, 10]. In India, from early times, related parts of herbal plants have been chose totreatexact ailments. Today, there is wide range of result in drugs resultant from plants. This is very importance for the confidence that herbal medicines are not dangerous and faithful compared with expensive synthetic drugs which have adverse effects. Natural antimicrobials can be resulting from animal tissues, plants, or microorganisms. The shortcomings of the drugs offered today, thrust the advance of new pharmacotherapeutic agents in medicinal plants. To find out the credible and progress the use of herbal medicine, it is basic to increase the study of medicinal plants that discover place in legends ^[11].

Erythrina indica Linn. (Fabaceae) is a prickly, medium-sized, deciduous tree normally mounting to 27 m. tall, found in Bengal and in southern India. It is usually known as Pangara in Marathi, Indian coral tree or tropical coral tree in English, Mandara in Hindi & Paribhadra in Sanskrit.

Particular parts of plant are used in predictable medicines as a antiseptic, collyrium, nervine sedative, anti-asthmatics, in ophthalmia, antiepileptic, and as an astringent. Bark is used in fever, rheumatism, liver ailment and wound heal^[12, 13].

Arka (*Calotropis procera*) a considerable drug of Ayurveda is known in this state from the earliest time. It is mentioned by the initial Hindu writers and the olden name of the plant which originates in the Vedic journalism was Arka alluding to the form of leaves, which was used in the sacrificial resources^[14]. It has been mostly used in the Unani, Sudanese, Arabic and Indian traditional medicinal system for the treatment of different diseases namely ulcers, leprosy, diseases of the spleen, piles, liver and abdomen^[15].

Ficus religiosa Linn. (Moraceae) generally known as 'Peepal tree' is a enormous extensively split tree with fibrous, heart shaped long tipped leaves on long slender petioles and purple fruits rising in pairs. The tree is regarded as a holy tree to both Buddhists as well as Hindus. It has got blessed, legendary and medicinal significance in Indian society since olden times^[16,17]. Bark give up stigmasterol, aspartic acid, campesterol, α -amyrin, isofucoesterol, tryptophan, lupeol, arginine, serine, leucine, threonine, tannic acid, n-hentricontanen, glycine, proline, tryosine, methionine, valine, isoleucine, n-nonacosane, hexa-cosanol n-octacosan and alanine^[18,19].

MATERIALS AND METHODS

Source of Plant Materials

The *Erythrina indica* leaf, *Calotropis procera* root & *Ficus religiosa* bark were collected from Samangad hill, Takuka-Gadhinglaj, Dist- Kolhapur (M.S.). The collected materials were inspected for their pathogenic infections. Healthy materials were selected after examining carefully. The materials were washed in running tap water for removing the surface contaminants. The washed materials were dried at room temperature for ten to fifteen days under shades. After drying the materials were powdered using blender^[20].

Chemicals and reagents

Methanol, Sulphuric Acid, Drangendroff's reagent, Molisch's reagent, Acetone.

Equipments

Soxhlet apparatus, Incubator, Digital balance, Bunsen burner, pH meter, Glass wares, blender & Magnetic stirrer.

Media

Nutrient Agar and Muller Hinton Agar Media

Organism:-

Two gram positive viz; *Bacillus cereus*, *Staphylococcus aureus* & two gram negative *Escherichia coli*, *Pseudomonas aeruginosa* are used for antimicrobial activity.

PREPARATION OF EXTRACTS^[21, 22]

Grinding of the Selected Plant Materials

After drying at 37°C for sufficient time the plant material was ground in a grinding made for the laboratory. Exposure to direct sunlight was avoided to prevent the loss of active components & the fine powder is collected.

Preparation of the Aqueous extracts

The *Erythrina indica* leaf was collected & air dried. After drying the materials were powdered using blender. Hundred grams of the air-dried powder plant materials was macerated for 7 days with 300 ml of sterile distilled water with continuous stirring. Then the aqueous extract was collected. The extract was first filtered through double layer muslin cloth & again filtered by using Whatman No 1 filter paper and the filtrate was evaporated to dryness. Then weighed and stored at 22°C in desiccators until further use. Similar procedure was used for aqueous extract of *Calatropsis procer* root & aqueous extract of *Ficus religiosa* bark. Then the dried powder was stored at 22°C in desiccators until further use.

Preparation of the Chloroform extracts

The *Erythrina indica* leaf was collected & air dried. After drying the materials were powdered using blender. Hundred grams of the air-dried powder plant materials was macerated for 7 days with 300 ml of chloroform with continuous stirring. Then the chloroform extract was collected. The extract was first filtered through double layer muslin cloth & again filtered by using Whatman No 1 filter paper and the filtrate was evaporated to dryness. Then weighed and stored at 22°C in desiccators until further use. Similar procedure was used for chloroform extract of *Calatropsis procera* root & chloroform extract of *Ficus religiosa* bark. Then the dried powder was stored at 22°C in desiccators until further use.

Preparation of the Methanol extracts

The *Erythrina indica* leaf was collected & air dried. After drying the materials were powdered using blender. Hundred grams of the air-dried powder plant materials was macerated for 7 days with 300 ml of methanol with continuous stirring. Then the methanolic extract was collected. The extract was first filtered through double layer muslin cloth & again filtered by using Whatman No 1 filter paper and the filtrate was evaporated to dryness. Then weighed and

stored at 22°C in desiccators until further use. Similar procedure was used for methanolic extract of *Calatropsis procera* root & methanolic extract of *Ficus religiosa* bark .Then the dried powder was stored at 22⁰c in desiccators until further use.

Preparation of the ethanol extracts (Continuous hot extraction-soxhelation process)

The *Erythrina indica* leaf was collected & air dried. After drying the materials were powdered using blender. Fifty grams of the air-dried powder plant materials was filled in a thimble & excreted by continuous hot extraction- soxhelation process by using 150 ml of methanol for 4 hr. Then the methanolic extract was collected & evaporated to dryness. Then weighed and stored at 22°C in desiccators until further use. Similar procedure was used for methanolic extract of *Calatropsis procera* root & methanolic extract of *Ficus religiosa* bark by using continous hot extraction soxhelation process .Then the dried powder was stored at 22⁰c in desiccators until further use

Preparation of Formulation

After collection of each three plants dried extract i.e. aqueous, chloroform, methanol (By maceration) & Methanol (By Soxhelation) the formulation was prepared in different concentration by using same solvent. The formulation& composition of polyherbal plants is as show in Table 1

Preparation of the tested organisms

Preparation of standard bacterial suspensions

The average number of viable, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*organisms per ml of the stock suspensions wasdetermined by means of the surface viable counting technique ^[23]. About (108-109) colony-forming units per ml was used. Each time, a fresh stock suspension was prepared; the experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

EVALUATIONS

Phytochemical analysis:-

The each extract obtained after maceration & soxhelation was subjected to various Phytochemical screening as per the standard procedure to reveals the presence of various active phytoconstituent^[24]. Phytochemical evaluation of each plant extract is given in table II.

Physicochemical parameters ^[25]

Preliminary evaluation of polyherbal formulations at different concentrations was carried out as follows:

Colour and odour

Colour and odour was examined by visual examination.

pH

The pH of various formulations was determined by using Digital pH meter. Different concentration of extract was dissolved in 100 ml of same solvent of extract and stored for two hours. The measurement of pH of each formulation was done in triplicate and average values were depicted in Table-III.

Stability studies

The stability studies were carried out for the prepared formulations at different temperature conditions (4° C, 25° C and 37° C) for 3 months.

Sample preparation:-

The extracts were weighed & prepared polyherbal formulation (F1 to F4) of different concentrated by using same solvent & 0.05 ml was used for activity studies.

Control preparation:-

The same solvents which are used for extraction process are used as control for the antimicrobial studies. 0.05 ml solvents are used for activity study.

Standard preparation:-^[26]

Amoxicillin serve as a standard control for antimicrobial activity.

(Amoxicillin 10µg/ml)

Preparation of medium and nutrient broth^[27]

Weighed about 0.4gm of nutrient soup and dissolved in 30ml of distilled water. Then the soup was hanged in each of test tube. The Muller Hinton agar medium was prepared which contain 9.7gm of MHA was hanged in 250ml of water. Then broth the medium and soup were for sterilization. After sterilization, the nutrient soup was permitted to cool and then the organisms

were inoculated for 4 hours. The MHA medium were poured in the petridish before cooling and allowed to solidify for about 3-4 hours.

Methodology

The antibacterial activity was determined according to the method described by Okeke (2001) with some modifications^[28]. *Bacillus cereus* culture was swabbed on the surface of sterile nutrient agar plate in triplicate. In agar plate five wells were prepared with the help of sterilized cork borer of 6mm diameter namely a,b,c,d,& e as show in fig. 1. The (a) center well is served as standard, (b) served as control, (c) served as 2% polyherbal extract, (d) served as 4% polyherbal extract & (e) served as 5% polyherbal extract . Each well was filled aseptically with 0.05ml std, control & polyherbal formulations by using micropipette. Similarly the antimicrobial activity of, *Staphylococcus aureus*, *Escherichia coli* & *Pseudomonas aeruginosa* was carried out. These plates were incubated at 37°C overnight for observation. The presence of inhibition was noted and compared with the standard. The susceptibility of the test organism to the tested plant extract was resolute by observing the zone of inhibition around each well.

Microbiological screening: -^[29, 30]

Antimicrobial activities of different extracts of polyherbal plant were evaluated by the agar well diffusion method.

Agar well diffusion method: -^[31]

Agar well-diffusion method was followed to find out the antimicrobial activity. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 8 hour old - broth culture of respective bacteria. Five wells (6mm diameter and about 2 cm a part) were made in each of these plates using sterile cork tool. Polyherbal extract of different concentration 2, 4 & 5 mg/ml was used. About 0.05 ml of polyherbal formulation is subjected into well by using micropipette and permitted to diffuse at room temperature for 2hrs. Control experiments comprising inoculums without plant extract were set up. The plates were incubated at 37°C for 18-24 h for bacterial pathogens. The diameter of the inhibition zone (mm) was measured and calculated. The readings were taken in three different fixed directions and the average values were recorded.

Determination of relative percentage inhibition^[32]

The relative percentage inhibition of the polyherbal extract with respect to standard control was calculated by using the following formula

$$\text{Relative percentage inhibition of the test extract} = \frac{100 \times (x-y)}{(z-y)}$$

Where,

x: total area of inhibition of the test extract

y: total area of inhibition of the solvent

z: total area of inhibition of the standard drug

The total area of the inhibition was calculated by using $\text{area} = \pi r^2$

Where, r = radius of zone of inhibition

Measurement of antimicrobial activity using Agar well diffusion Method

The antimicrobial activity of different formulated extract of polyherbal plants was evaluated according to their zone of inhibition against various pathogens and the results (zone of inhibition) were compared with the activity of the standards, viz. Amoxicillin antibiotic. The results revealed that the extracts are potent antimicrobials against all the microorganisms studied. Among of these different formulations the formula F4 showed high degree of inhibition.

The antimicrobial activity of different polyherbal ointment is listed in Table IV.

RESULTS AND DISCUSSION

This present study shown that the selected three herbs that are *Erythrina indica* leaf, *Calatropsis procera* root & *Ficus religiosa* bark was made to create a different concentration of polyherbal formulation, and to evaluate for its physical parameter, and to compare its antimicrobial activity with a standard antibiotic like Amoxicillin. These three plants are extracted by maceration process using a aqueous, chloroform & methanol solvents & again continuous hot extraction (soxhlation) process by methanol solvent and the Phytochemical screening was done. Phytochemical screening established the presence of various phytoconstituent like alkaloids, carbohydrate, glycosides, flavanoids, amino acids, steroid, saponin, protein, phenolic compound and tannins.

In the present work, the extract was studied for physical parameter, phytochemical constituents & the different concentrated polyherbal formulations in vitro antimicrobial activity was compared with standard antibiotic like Amoxicillin. The physical parameters were within the acceptable range. The stability studies were carried out and inferred that the formulations showed no signs of instability. The antimicrobial activity of prepared formulations were compared with antibiotic like Amoxicillin using selected species of gram positive bacteria such as *Bacillus cereus*, *Staphylococcus aureus* & gram negative bacteria such as *Escherichia coli*,

Pseudomonas aeruginosa and it was showed that formulation like F4 showed greater antimicrobial activity against given micro-organism as compared to antibiotic like Amoxicillin. It was also seen that the by increasing in a concentration of extract the polyherbal formulations show an increase in antimicrobial activity. The antimicrobial activity of polyherbal formulation is due to the presence of flavanoids and tannins. Hence the study concludes that the methanol extract by continuous hot extraction (soxhlation) process is an antimicrobial activity, it is also efficient antiseptic purpose with antimicrobial activities can be formulated from the methanolic plant extracts(soxhlation) of *Erythrina indica* leaf, *Calatropsis procera* root & *Ficus religiosa* bark which can also be used for wound healing and various skin infections.

Table 1 Formulation of *Erythrina indica* leaf, *Calatropsis procera* root & *Ficus religiosa* bark for antimicrobial activity

Plants	Extract by Maceration Process			Extract by Soxhlation Process
	Aqueous(F1)	Chloroform(F2)	Methanol(F3)	Methanol(F4)
<i>Erythrina indica</i> leaf	2%	2%	2%	2%
<i>Calatropsis procera</i> root	4%	4%	4%	4%
<i>Ficus religiosa</i> bark	5%	5%	5%	5%

Table II: Phytochemical screening of the extract:-

A) Phytochemical screening of the Aqueous extract of *Erythrina indica* leaf, *Calatropsis procera* root & *Ficus religiosa* bark

Sr.No.	Name Of Test	Aqueous Extract of <i>Erythrina indica</i> leaf	Aqueous Extract of <i>Calatropsis procera</i> root	Aqueous Extract of <i>Ficus religiosa</i> bark
1	Alkaloids	++	++	-
2	Glycosides	++	+	+
3	Carbohydrates	+	-	-
4	Flavonoids	++	-	++
5	Tannins	+	-	+
6	Steroids	-	++	+
7	Amino acid			
8	Saponins	+	-	-
9	Proteins	+	+	+
10	Phenolic compound	+	-	++

+ - -

B) Phytochemical screening of the Chloroform extract of *Erythrina indica* leaf, *Calatropsis procera* root & *Ficus religiosa* bark

Sr.No.	Name Of Test	Chloroform Extract of <i>Erythrina indica</i> leaf	Chloroform Extract of <i>Calatropsis procera</i> root	Chloroform Extract of <i>Ficus religiosa</i> bark
1	Alkaloids			
1	Glycosides	++	++	++
2	Carbohydrates	-	+	-
3	Flavonoids	-	-	+
4	Tannins	++	+	++
5	Steroids	+	+	++
6	Amino acid	+	++	+
7	Saponins	+	+	-
8	Proteins	-	+	-
9	Phenolic compound	-	-	+
10		-	-	-

C) Phytochemical screening of the Methanolic extract of *Erythrina indica* leaf, *Calatropsis procera* root & *Ficus religiosa* bark

Sr. No.	Name Of Test	Methanolic Extract of <i>Erythrina indica</i> leaf	Methanolic Extract of <i>Calatropsis procera</i> root	Methanolic Extract of <i>Ficus religiosa</i> bark
1	Alkaloids	+++	++	++
2	Glycosides	-	-	+
3	Carbohydrates	+	-	-
4	Flavonoids	++	+	++
5	Tannins	+	+	+++
6	Steroids	++	++	+
7	Amino acid			
8	Saponins	+	+	-
9	Proteins	+	+	-
10	Phenolic compound	+	-	+
		+	-	-

D) Phytochemical screening of the Methanolic extract (Soxhalation) of *Erythrina indica* leaf, *Calatropsis procera* root & *Ficus religiosa* bark

Sr.No.	Name Of Test	Methanolic Extract of <i>Erythrina indica</i> leaf	Methanolic Extract of <i>Calatropsis procera</i> root	Methanolic Extract of <i>Ficus religiosa</i> bark
1	Alkaloids	+++	++	++
2	Glycosides	+++	-	+
3	Carbohydrates	+	+	-
4	Flavonoids	+++	++	++
5	Tannins	+	+	++
6	Steroids	++	+++	++
7	Amino acid			
8	Saponins	+	+	-
9	Proteins	+	+	-
10	Phenolic compound	+	-	+
		+	+	-

+Present, - absent, ++ appreciable present, +++ very appreciable present.

Table III: Physicochemical evaluation of different concentrated polyherbal formulations

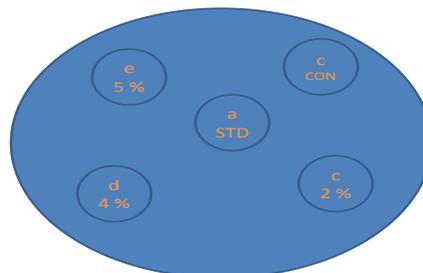
Sr.No.	Physicochemical Properties	F1	F2	F3	F4
1	Color	Pale Brown	Pale Brown	Pale Brown	Pale Brown
2	Odour	Characteristics	Characteristics	Characteristics	Characteristics
3	PH	5.72	6.12	6.91	7.02
4	Storage (4 °C,24 °C,36 °C)	Stable	Stable	Stable	Stable
5	Percentage yield of Aqueous extract		Chloroform extract	Methanol extract	Methanol extract (Soxhellation)
	<i>Erythrina indica</i> leaf	13.9	12.4	14.2	14.8
	<i>Calatropsis procera</i> root	12.8	9.8	13.2	13.4
	<i>Ficus religiosa</i> bark	13.2	11.3	13.4	13.6

Table IV: Antimicrobial activity of different concentrated polyherbal formulation

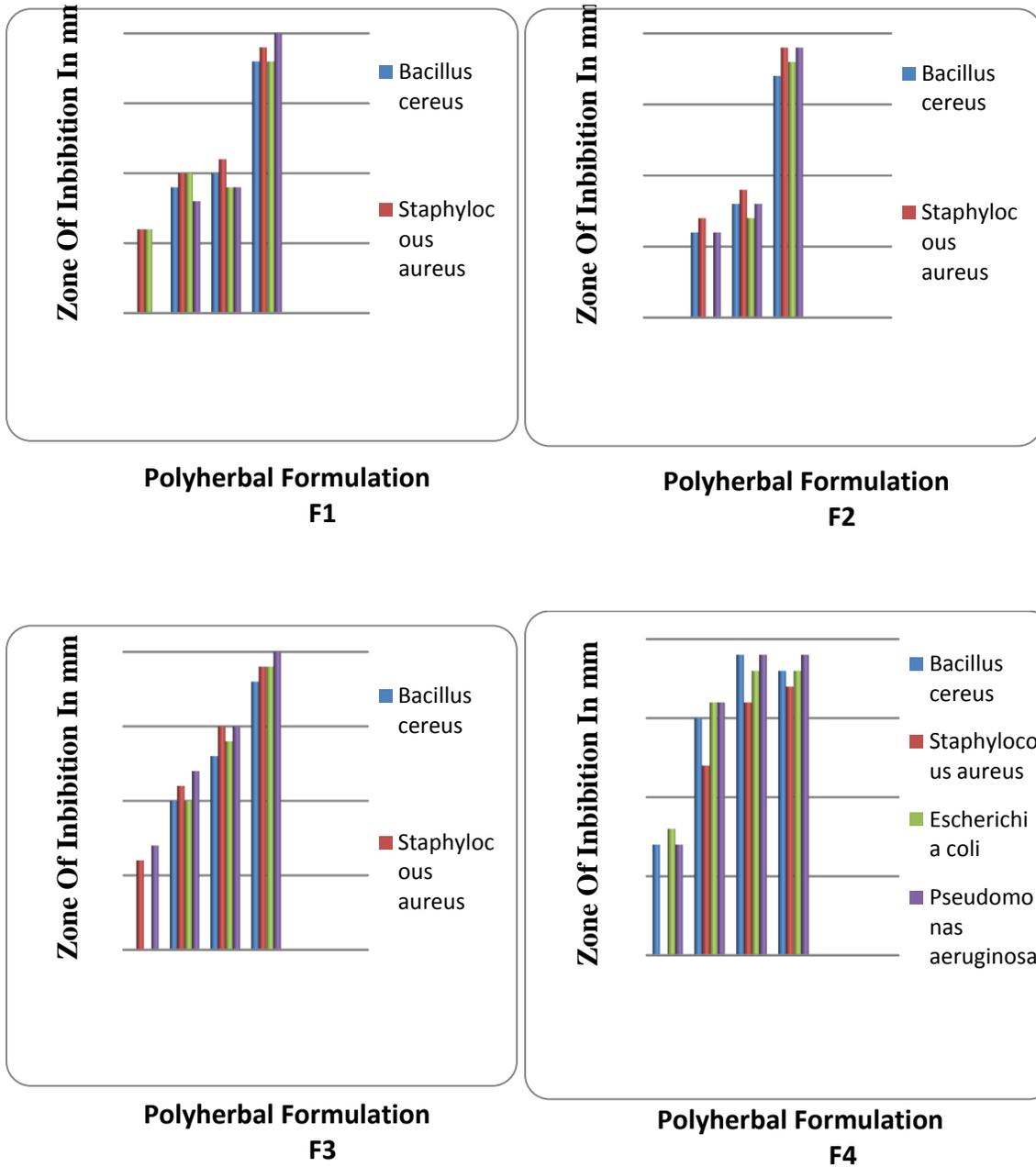
Sr.No	Polyherbal Formulation	Concentration	Inhibited Zone Diameter in mm			
			<i>Bacillus cereus</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
1	F1	2%	-	6	6	-
		4%	9	10	10	8
		5%	10	11	9	9
		Standard	18	19	18	20
		Control	-	-	-	-
2	F2	2%	-	-	-	-
		4%	6	7	-	6
		5%	8	9	7	8
		Standard	17	19	18	19
		Control	-	-	-	-
3	F3	2%	-	6	-	7
		4%	10	11	10	12
		5%	13	15	14	15
		Standard	18	19	19	20
		Control	-	-	-	-
4	F4	2%	7	-	8	7
		4%	15	12	16	16
		5%	19	16	20	19
		Standard	18	17	18	19
		Control	-	-	-	-

Figure 1

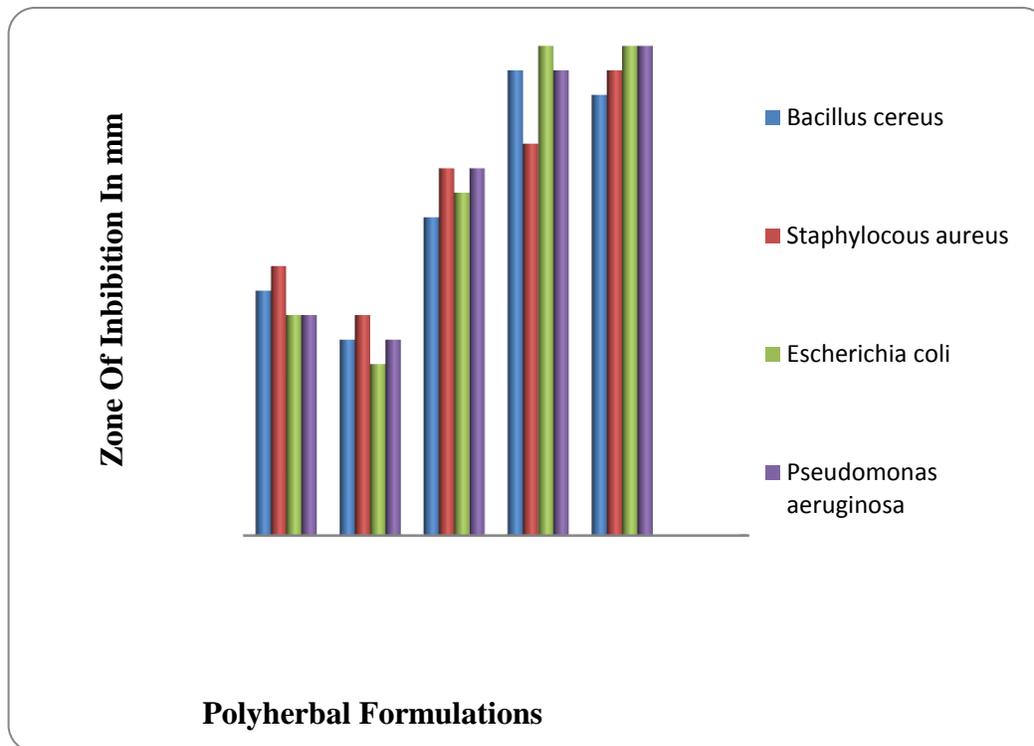
Schematic diagram showing use of methodology for antimicrobial activity of different concentrated formulation



Graphical representation of different concentrated polyherbal formulation



Graphical Representation of Comparision of 5% Formulations F1 to F4 with standard



A.E.= Aqueous Extract, C.E.= Chloroform Extract, M.E.=Methanolic Extract &M.E.(S)= Methanolic Extract (By Soxhlet process)

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