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Research Article

Microbiology for Traditional Medicine

Plasmid-Curing, Antimicrobial, Antioxidant Properties and Phytochemical Analysis of Medicinal Plants from North East India

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Abstract: The medicinal importance of plants is in their bioactive substances which exert definite physiological action on the human body. In the present investigation antimicrobial activities of *Garcinia pedunculata*, *Phlogacanthus thyrsiformis*, and *Ziziphus mauritiana* were studied against microbial strains using agar disc diffusion. *In-vitro* phytochemical screening for chloroform, isoamyl alcohol and water extracts of parts of plants was performed. For MIC (minimum inhibitory concentration), grid method was used. The antioxidant activity of the plant extracts was studied using the ferric reducing antioxidant method and bioautography was studied using Thin Layer Chromatography (TLC). *Garcinia pedunculata* and *Phlogacanthus thyrsiformis* extracts showed highest antimicrobial activity. *In-vitro* phytochemical screening for chloroform, isoamyl alcohol and water extracts of parts of plants showed positive results for alkaloids, saponins, steroids, triterpenes, flavonoids and diterpenes. The MIC value of *Garcinia pedunculata*, *Phlogacanthus thyrsiformis* and *Ziziphus mauritiana* was 2560, 1280 and 2560 µL, respectively. The antioxidant activity revealed that there was an increase in absorbance with the increase of sample concentration. In Thin Layer Chromatography-Bioautography, chloroform extract of *Garcinia pedunculata* and *Ziziphus mauritiana* showed activity with zones of inhibition on bioautograms. The chloroform extract of *Ziziphus mauritiana* was found to be effective with curing efficiency for *E. coli* K12 (RP4), *E. coli* (pBR322) and *E. coli* (pRK2013) 62%, 57% and 49% respectively. Petroleum ether extract of *Phlogacanthus thyrsiformis* cured *E. coli* K12 (RP4), *E. coli* (pBR322) and *E. coli* (pRK2013) at 38%, 42% and 35% curing efficiencies respectively. This is the first report of plasmid curing by using chloroform *Ziziphus mauritiana* and petroleum ether extract of *Phlogacanthus thyrsiformis*. The present investigation has revealed applications and significance of plant extracts of *Garcinia pedunculata*, *Phlogacanthus thyrsiformis*, and *Ziziphus mauritiana* as plasmid curing, antimicrobial and antioxidant agents to control infections and spread of antibiotic resistance in pathogenic bacteria.

Keywords: Antimicrobial, Phytochemical, Plasmid curing, Antioxidant, Bioautography

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1. INTRODUCTION

Plants are known to produce diverse bioactive substances of therapeutic value. Plants such as vegetables, fruit, spices, medicinal herbs, etc., have been used to cure many diseases since ancient time. Synthetic drugs which are readily available and highly effective in curing various diseases have some disadvantages and are more harmful as compared to the traditional folk medicines. Diversity of secondary metabolites isolated from plants have shown that these compounds have anticancer, antibacterial, analgesic, anti-inflammatory, antitumor, antiviral and anti plasmid activities[1,2]. The medicinal plants should be studied for their properties, safety, and efficacy and for new potent antimicrobial compounds and fractions. During the past few years, there has been a dramatic increase in microbial resistance to antimicrobial agents [3,4] which has led to repeated use of antibiotics[5]. Plants are the most common and important sources of potentially valuable new drugs. There is, therefore, an urgent need to study the biological properties of additional medicinal plants to develop new drugs. The medicinal plants and their uses are shown in Table 1[6]. A wide range of medicinal plant parts are used as extracts for raw drugs. The medicinal value of the plants lies in the bioactive substances which produce definite physiological action on the human body. Different parts of plants are used including root, stem, flower, fruit, twigs exudates and modified plant organs[7]. The phytochemical compounds include flavonoids, phenols and phenolic glycosides, saponins and cyanogenic glycosides, stilbenes, tannins, alkaloids, amines, betalains, terpenoids and some other endogenous metabolites [1,8]. Plants contain many beneficial phytochemicals supplements that are essential for the human body acting as natural antioxidants. The consumption of fruits and vegetables has been linked with several health benefits, [9]. *Garcinia pedunculata* (*Amlavetasa*) is an excellent cardiac stimulant, laxative and having digestive capacity used in Ayurved since 1500 BC. Its classical uses are in the treatment of cough, asthma, bronchitis, piles, intestinal worms, bloating, splenomegaly, anaemia, heart diseases, anorexia, indigestion, constipation, abdominal pain due to digestion imbalance and bloating, treatment of epilepsy, toxic conditions, dysuria, ascites, malabsorption syndrome, etc. It is found and harvested in North Eastern states of India like Assam, Manipur and Shillong[10]. The dried fruits of *Ziziphus mauritiana* are used as anodyne, anticancer, pectoral, refrigerant, sedative, stomachache, styptic and tonic. They are considered to purify the blood and aid digestion. The root is used in the treatment of dyspepsia. A decoction of the root has been used in the treatment of fevers[11]. *Phlogacanthus thyrsiformis* is very commonly used as a folk medicine in Assam. It is used as an antihistamine. It is also used for curing cough and cold, chronic bronchitis, asthma, and rheumatism. Different parts of the plant have been used as an antiseptic and as a good insecticide[12]. The aim of this study is to screen these medicinal plants for phytochemical compounds; antimicrobial activity; minimum inhibitory concentration; antioxidant, plasmid curing activity and bioautography.

2. MATERIALS AND METHODS

2.1. Collection of Plant Materials

Fresh leaves, inflorescence bark, seeds, and roots of three different plants viz. *Garcinia pedunculata, Phlogacanthus thyrsiformis* and *Ziziphus mauritiana* were collected from different plant nurseries situated in Assam, North East India. Leaves and fruits of *Garcinia pedunculata* and *Ziziphus mauritiana* and inflorescence of *Phlogacanthus thyrsiformis* were authenticated by 'Botanical Survey of India, Pune, Maharashtra, India'. All the plant materials were cleaned, air dried under shade and powdered with a dry mill.

2.2. Preparation of Plant Extracts

Powdered leaves of *Garcinia pedunculata* and *Ziziphus mauritiana* and inflorescence of *Phlogacanthus thyrsiformis* (Figure1) were extracted by differential extraction using Soxhlet apparatus. Plant extraction was done using cold water, hot water, and organic solvents viz., chloroform, n-hexane, benzene, isoamyl alcohol, diethyl ether and petroleum ether. Solvents were used according to eluotropic series based on polarity. The extracts were filtered and concentrated to dryness under reduced pressure on a rotary evaporator (Heidolph-Germany). After evaporation of the solvents, extracts were dissolved in Dimethyl sulfoxide (DMSO) and were evaluated for their potential.

2.3. Antimicrobial Study[13]

The test organisms selected for antimicrobial activity were viz., *Bacillus cereus*, *Bacillus subtilis*, *E. coli*, *Staphylococcus aureus*, *Serratia marcescens*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Aspergillus niger, Aspergillus flavus* and *Candida albicans*. The bacterial cultures were maintained on nutrient agar (NA) slants while the fungal cultures viz., *Aspergillus niger* and *Aspergillus flavus* were maintained on Sabouraud's dextrose agar (SDA) slants. The bacterial cultures were inoculated into a sterile nutrient broth and for the fungal cultures, the spores were scraped and inoculated into sterile Sabouraud's dextrose broth. The flasks were kept for overnight incubation on a rotary shaker at 37°C. The cell density was adjusted with 0.5 McFarland turbidity standards to obtain final concentration of 10^6 cells/ mL and 10^5 spores/mL, respectively. For antibacterial and antifungal activity, 0.1 mL of inoculum suspension was spread on NA and SDA plates respectively and the plates were kept in the refrigerator for 30 min for diffusion. Disc diffusion method was used. 10 µL of plant extracts were added onto the disc. Amikacin was (10 µg /mL) used as a positive control and dimethyl sulfoxide (DMSO) as a negative control. The plates were incubated at 37°C for 24 hrs and the zones of inhibition were measured.

2.4. Phytochemical Screening

The tests for phytochemical compounds of the plant extracts viz., steroids, triterpenoids, alkaloids, tannins, flavonoids, diterpenes, glycosides and saponins were studied as per the standard methods[11].

2.5. Minimum Inhibitory Concentration Assay (MIC)[14]

For MIC, grid method was used where the plant extracts were diluted from 40 to 5120 µL/ml. The organisms selected were viz., *E. coli*, *S. aureus*, *P. vulgaris*, *K. pneumoniae*, *P. aeruginosa*, *B. cereus* and *S. marcescens*. Cultures were spread from low to high concentration of plant extracts on nutrient agar plates containing plant extract in discs. The plates were incubated at 37°C for 24 hrs and the MIC was recorded as

the lowest concentration of the plant extract where the growth of the organism was inhibited.

2.6. Antioxidant Activity

The antioxidant activity was studied by ferric reducing antioxidant method[15]. The concentration of plant extracts viz., 10, 25, 50 and 100 mg in 1 mL of ethanol was prepared and mixed with 2.5 mL of phosphate buffer (2 M, pH 6.6). following which 2.5 mL of potassium ferricyanide (10 g/L) was added. The mixture was incubated at 50^{0}C for 20 min and 1.5 mL of trichloroacetic acid (TCA) (100 g/L) was added and centrifuged at 3000 rpm for 10 min. The supernatant 0.5 mL was mixed with 1 mL distilled water (DW) and 0.5 mL of ferric chloride. The absorbance was measured at 665 nm. The increase in absorbance indicated the antioxidant activity of the plant extracts.

2.7. Thin Layer Chromatography (TLC) – Bioautography

The TLC-Bioautography of plant extracts was performed [16,17]. TLC plates were spotted with plant extracts and developed in a solvent system [chloroform: ethyl acetate: formic acid (10:8:2)]. The plates were then dried under an airstream. Inoculum was prepared in nutrient broth (NB) using McFarland turbidity standard. The TLC plates were placed in sterile petri plates, covered with 4.5 mL of inoculum, and incubated at 37^{0}C for 15 hrs. After incubation, the plates were sprayed with an aqueous solution of phenyl tetrazolium chloride (2 mg /mL) and incubated at 37°C for 1 h[16,17]. The clear zones on the chromatogram indicated growth inhibition.

2.8. Standard Microbial Cultures

The standard antibiotic resistant reference plasmids viz., *E. coli* K12 (RP4), *E. coli* (pRK2013) and *E. coli* (pBR322) were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India.

2.9. Plasmid Curing

The plasmid curing was performed as described earlier[18]. The cultures viz., *E. coli* K12 (RP4), *E. coli* (pRK2013) and *E. coli* (pBR322) were grown in the presence of plant extracts at the sub-inhibitory concentration for 24 h at 37^{0}C and plated on Luria agar (LA) plates [LA (g/L): casein enzymatic hydrolysate:10, yeast extract:5, sodium chloride:5, agar:15. pH has to be 7.0] to obtain well isolated colonies. The isolated colonies were inoculated on the LA plate to get the master plate. The colonies from the master plate were replica plated on LA containing antibiotics. The colonies formed on the LA master plate which failed to grow in presence of an antibiotic, were considered as putative cured derivatives. The percentage of curing efficiency was expressed in *E. coli* K12 (RP4) (Ap^r, Km^r, Tc^r), *E. coli* (pRK2013) (Ap^r, Km^r) and *E. coli* (pBR322) (Ap^r, Tc^r) as the number of colonies with cured phenotype per 100 colonies tested. Antibiotic sensitive cured colonies were also tested for loss of resistance to antibiotics by disc diffusion assay. The experiments were performed in triplicates.

3. RESULTS AND DISCUSSION

3.1. Antimicrobial Activity

P. thyrsiformis leaves having secondary metabolites like flavonoids, saponins, and tannin have been shown to be responsible for the antimicrobial activity of plants. Methanolic extracts showed maximum antibacterial properties against *S. typhimurium* and *S. enterica*[12]. *Phlogacanthus thyrsiformis* showed moderate potential antibacterial against *Bacillus subtilis*[19]. In the present study, benzene extract of *Garcinia pedunculata* had the highest activity against *P. aeruginosa,* with zone of inhibition 20 mm. Petroleum ether extract of *P. thyrsiformis* had antibacterial activity against *E. coli with a zone* of inhibition 24 mm. The antifungal activity of *P. thyrsiformis* extract prepared in chloroform, cold water, isoamyl alcohol and diethyl ether showed significant activity against *Aspergillus niger* with zone of inhibition 4, 7, 13 and 10 mm, respectively (Table 2). The methanol and ethyl acetate leaves extracts of *P. thyrsiformis* displayed antifungal activity against *A. niger* and *C. albicans*[20]. The chloroform extract of *Ziziphus mauritiana* showed the highest activity against *P. aeruginosa,* with zone of inhibition 19 mm. The antibacterial activity of *P. thyrsiformis* inflorescence extract, *G. pedunculata* and *Z. mauritiana* leaves extract against *Proteus vulgaris* and *E. coli* are shown in Figure 2 and 3, respectively. The antifungal activity of *Ziziphus mauritiana* plant extract prepared using chloroform, n-hexane, petroleum ether and isoamyl alcohol showed zone of inhibition against *Aspergillus niger* and the highest activity was found by isoamyl alcohol extract with 16 mm zone of inhibition (Table 2). The antifungal activity of *G. pedunculata* leaves extract against *Aspergillus flavus* is shown in Figure 4. The crude methanolic extract of *Ziziphus mauritiana* leaves were observed to be rich in phytochemical constituents and had significant levels of antioxidant and antimicrobial activities. The leaf extract did not show a significant level of antibacterial activity against Gram negative bacteria[21]. Leaves extracts of *Z. mauritiana* possessed antimicrobial activity against *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhimurium* and *E. coli* with the highest total antioxidant capacity[22]. In the present study, all the three plant extracts using different solvents showed antimicrobial activity when compared with Amikacin. The antibacterial activity of Amikacin as a positive control against the test organisms is shown in Table 2. There is a report where five plant extracts were investigated against *B. cereus*, *S. aureus*, *E. coli*, *P. aeruginosa* and *S. typhi*. The ethanolic extracts of *Punica granatum, Syzygium aromaticum*, *Zingiber officinale*s and *Thymus vulgaris* were effective with variable efficiency against the bacterial strains at concentration of 10 mg /mL, while the extract of *Cuminum cyminum* was only effective against *S. aureus*[15]. A Study has also been done where methanolic extracts of *Berberis vulgaris*, *Cassia angustifolia*, *Cinnamomum cassia*, *Cistus monspeliensis*, *Nigella sativa*, *Punica granatum*, *Rhus tripartita*, *Withania frutescens* and *Zingiber officinale* were tested for antibacterial activity. The plant extracts showed inhibition zones ranging from 6 to 23 mm and MIC ranged from 0.1 to 12.8 mg/mL[23].

3.2. Phytochemical Analysis

The plant extracts of *Garcinia pedunculata*, *Phlogacanthus thyrsiformis* and *Ziziphus mauritiana* revealed the presence of active phytochemical compounds viz., alkaloids, saponins, steroids, triterpenes, flavonoids and diterpenes. The results of phytochemical compounds are shown in Table 3. The qualitative and quantitative phytochemical analysis of *Moringa concanensis* has been reported[24] which showed the presence of alkaloids, flavonoids, phenol and carbohydrates[25]. The phytochemical analysis of plants viz., *Garcinia indica*; *Jatropha*

curcas; *Nigella sativa*; *Levisticum officinale*; *Dracaena loureiri*; *Woodfordia fruticosa*; *Vaccinium macrocarpon*; *Foeniculum vulgare*; *Sapindus saponaria* and *Annona squamosa* has been reported[26]. Phytochemical screening of *G. pedunculata* revealed the occurrence of compounds viz., alkaloids, carbohydrates, saponin, phenolic compounds and proteins along with fixed oils and fats, glycosides, and amino acids[27].

3.3. Minimum Inhibitory Concentration Assay

For *Garcinia pedunculata*, the MIC value was found to be 2560 µL. For *Phlogacanthus thyrsiformis*, the MIC value was 1280 µL, except for *Bacillus cereus* whose growth was observed at 5120 µL. For *Ziziphus mauritiana*, the MIC value was 2560 µL, except for *Serratia marcescens* whose growth was observed at 5120 µL. The results of MIC of the plant extracts are shown in Table 4.

3.4. Antioxidant Activity

The absorbance was found to increase with the increase of concentration of three plant extracts (10 to 100 mg/mL) which indicated increased reducing power; thus, proving the antioxidant property (Figure 5). In the present investigation, *Phlogacanthus thyrsiformis* has shown greater antioxidant activity than the standard (H_2O_2) as well as *Garcinia pedunculata* and *Ziziphus mauritiana.* At 100 mg/ml concentration, *P. thyrsiformis* has shown maximum antioxidant activity. The leaves and flowers of *P. thyrsiformis* are good sources of natural antioxidants which are useful in treating the diseases associated with oxidative stress[28]. Aqueous as well as methanol extract of *P. thyrsiformis* possessed versatile free radical scavenging activity[29]. Phenolic compounds in the crude extracts of *Phlogacanthus pulcherrimus* contributed to strong antioxidant activity; ethyl acetate and dichloromethane extracts of *P. pulcherrimus* showed antioxidant and antiproliferative activity[30] (Poeaim, 2016). Total antioxidant activity in terms of IC50 value was found to be the highest (11.61) in *Garcinia pedunculata* as compared to other species *Garcinia cowa*, *Garcinia lanceifolia* and *Garcinia xanthochymus* of Assam[31]. Leaf methanol extracts of nine *Garcinia* species from the Western Ghats exhibited remarkable *in-vitro antioxidant* activity against various free radicals due to the presence of high phenolic and flavonoid contents[32]. The *in-vitro* evaluation of antioxidant activity of methanol extracts of medicinal plants has been studied, where the methanol extracts of *C. coggygria* and *R. damascena* exhibited potent antioxidant activity by 1, 1-diphenyl-2-picrylhydrazyl (DPPH), ferric thiocyanate (FTC) and thiobarbituric acid (TBA) methods[33]. There is also a report on the *in-vitro* and *in-vivo* antioxidant activity of the butanol extract from the stem of *Ephedra alte*[34].

3.5. Bioautography

In TLC-Bioautography, the plant extracts viz., *Garcinia pedunculata*, *Phlogacanthus thyrsiformis* and *Ziziphus mauritiana* prepared in chloroform showed the presence of one or more active compounds. The *Garcinia pedunculata* plant extract showed activity against *K. pneumoniae* with R_f value 0.23. The *Ziziphus mauritiana* plant extract showed activity against *S. marcescens* with R_f value 0.76. The *Phlogacanthus thyrsiformis plant* extract did not show any activity against the test organisms. Earlier report on TLC-Bioautography detection and biological activity of antifungal compounds from medicinal plant *Acorus calamus* L., where clear zones of inhibition of fungal growth corresponding to the positions producing large inhibitory zones at R_f 0.7 for fungal strains *F. oxysporum*, *F. solani*, *C. gloesporioides*, *B. cinerea* and *A. solani* [35]. Also, work has been reported on antimicrobials of medicinal plants viz., clove, cinnamon, datura and tulsi, where the maximum zone of inhibition of clove, cinnamon, datura and tulsi were observed at R_f 0.639, 0.147, 0.803 and 0.263 respectively[36]. Study has been done on TLC screening and evaluation of antioxidant, antibacterial activity of *Onopordon macrocephalum* by bioautography[37]. Although there have been studies on phytochemical analysis of plant extracts, this is the first report on the phytochemical, antimicrobial, antioxidant activity and bioautography studies of medicinal plants viz., *Garcinia pedunculata*, *Phlogacanthus thyrsiformis* and *Ziziphus mauritiana*.

3.6. Plasmid Curing

The antiplasmid activity of plant extracts in different solvents was tested against the reference plasmids (Table 5). Among three extracts which were tested, chloroform extract of *Ziziphus mauritiana* demonstrated the maximum curing activity as compared to the other two, against plasmid harbouring reference strains. The chloroform extract of *Ziziphus mauritiana* cured *E. coli* K12 (RP4), *E. coli* (pBR322) and *E. coli* (pRK2013) at 62%, 57% and 49% curing efficiencies, respectively (Table 5). The petroleum ether extract of *Phlogacanthus thyrsiformis* was found to be effective for curing *E. coli K12* (RP4), *E. coli* (pBR322) and *E. coli* (pRK2013) with 38%, 42% and 35% efficiencies, respectively. The plasmid curing efficiency was less with benzene extract of *Garcinia pedunculata,* which cured *E. coli* K12 (RP4), *E. coli* (pBR322) and *E. coli* (pRK2013) at 10%, 9% and 12% curing efficiencies, respectively. The plasmid cured standard strains when tested for resistance/sensitivity to antibiotics by the disc diffusion assay, were found to be sensitive to the respective antibiotics. The elimination of plasmid-determined antibiotic resistance in pathogenic strains of bacteria is of great functional importance, both in the treatment of microbial infections and in microbial genetics. Few articles in the recent years have revealed about plasmid curing in bacteria after treatment with certain plant extracts[38]. However, such an activity regarding plant extracts of *Garcinia pedunculata, Phlogacanthus thyrsiformis* and *Ziziphus mauritiana* has been reported for the first time. Though Plasmids can spontaneously be lost the frequency of spontaneous mutation in bacteria is less than one in 10^6 cells[39]. Mutagenic activity of any compound can be harmful especially in clinical applications. In the present study, plasmid curing by plant extracts of *Garcinia pedunculata*, *Phlogacanthus thyrsiformis* and *Ziziphus mauritiana* is at much higher frequency (9-62%), Hence they can be authentic plasmid curing agents. Sub-inhibitory concentrations of curing agents were used in the present research. It means that to these concentrations of compounds, the bacteria were already resistant. Therefore, it is projected that bacteria may not ever develop any way to counter the plasmid curing ability of the compounds present in the plant extracts of *Garcinia pedunculata, Phlogacanthus thyrsiformis* and *Ziziphus mauritiana*. The ability of these plant extracts to cure plasmid encoded antibiotic resistance in standard *E. coli* plasmid containing strains is significant particularly since the *E. coli* strains are known to act as a reservoir of antibiotic resistance genes. *Garcinia pedunculata, Phlogacanthus thyrsiformis* and *Ziziphus mauritiana* medicinal plants are used in the traditional Indian medicine system for hundreds of years. Hence, they are unlikely to cause any toxic / mutagenic / carcinogenic /

teratogenic effects on individuals. Thus, such root extracts as plasmid curing agents have tremendous advantage over conventional curing agents such as acriflavine, ethidium bromide, acridine orange which are known to be toxic, mutagenic, and carcinogenic. Further, these plant extracts can be used in basic research to obtain plasmid less derivatives and thus to determine plasmid encoded phenotypes in laboratory studies.

Table 1: Medicinal plants and their uses

Scientific name	Common name	Plant part used	Uses
Garcinia pedunculata	Kuji thekera	Dried fruits, leaves	Digestive and dysenteric properties[27]
Phlogacanthus thyrsiformis	Titaful	Inflorescence	Vermicide and also remedy of cough[12]
Ziziphus mauritiana	Bogori	Root, leaves, fruits	Bleeding disorders, fever, burning sensation[11]

Table 2: Antimicrobial activity of plant extracts against the test organisms

Plant extracts	Zone of inhibition in mm									
	E. coli	*S. marcescens*	*B. cereus*	*B. subtilis*	*P. aeruginosa*	*K. pneumonia*	*S. aureus*	*P. vulgaris*	*A. niger*	*A. flavus*
***G. pedunculata* (Leaves)**										
Chloroform	7±0.00	15±0.01	18±0.00	5±0.04	15±0.01	10±0.00	2±0.00	10±0.02	-	13
Isoamyl alcohol	2±0.01	10±0.01	15±0.00	4±0.01	14±0.02	12±0.00	12±0.03	6±0.03	-	-
Benzene	11±0.02	17±0.01	13±0.00	6±0.00	20±0.01	9±0.00	10±0.01	10±0.03	-	-
Hexane	2±0.02	4±0.00	4±0.02	10±0.01	10±0.00	10±0.02	11±0.00	9±0.01	-	-
Petroleum ether	12±0.00	15±0.00	11±0.01	7±0.00	10±0.01	7±0.00	12±0.00	13±0.01	-	-
Diethyl ether	9±0.00	16±0.02	8±0.03	3±0.02	16±0.01	5±0.00	15±0.02	9±0.01	-	-
***P. thyrsiformis* (Inflorescence)**										
Chloroform	15±0.00	11±0.00	-	11±0.00	8±0.02	19±0.00	-	16±0.02	4±0.01	-
Isoamyl alcohol	21±0.02	6±0.00	7±0.00	6±0.02	3±0.01	15±0.00	5±0.00	13±0.01	13±0.04	-
Benzene	19±0.00	2±0.01	2±0.00	1±0.00	11±0.02	-	13±0.02	7±0.00	-	-
Hexane	20±0.00	2±0.01	10±0.02	3±0.00	9±0.00	13±0.02	1±0.00	-	-	-
Petroleum ether	24±0.04	9±0.03	2±0.00	3±0.00	16±0.01	8±0.00	5±0.02	11±0.03	-	-
Diethyl ether	22+0.01	8±0.02	3±0.02	5±0.01	9±0.03	11±0.00	6±0.02	-	10±0.00	-
Hot water	11±0.00	-	10±0.01	7±0.00	3±0.00	13±0.01	-	4±0.00	-	-
Cold water	12±0.02	8±0.00	6±0.00	3±0.00	4±0.00	2±0.00	-	5±0.03	7±0.02	-
***Z. mauritiana* (Leaves)**										
Chloroform	14±0.01	17±0.01	16±0.02	-	19±0.01	4±0.00	-	15±0.00	10±0.00	-
Isoamyl alcohol	8±0.03	11±0.01	9±0.00	-	14±0.00	2±0.01	-	-	16±0.00	-
Benzene	2±0.01	-	-	-	4±0.00	-	-	-	-	-
Hexane	5±0.00	-	-	-	8±0.02	-	-	-	9±0.00	-
Petroleum ether	11±0.03	5±0.00	-	-	11±0.00	2±0.01	-	-	5±0.00	-
Diethyl ether	12±0.01	3±0.00	-	-	9±0.01	1±0.00	-	-	-	-
Cold water	6±0.00	10±0.01	5±0.00	-	12±0.03	7±0.00	-	13±0.00	-	-
Amikacin (Positive control)	45±0.03	30±0.03	45±0.04	40	25±0.00	30±0.01	40±0.01	35±0.01	30±0.00	

Table 3: Biochemical analysis of the plant samples

Plant extract	Biochemicals							
	Alkaloid	Steroid	Tannin	Triterpenoid	Flavonoid	Diterpene	Glycoside	Saponin
Garcinia pedunculata	-	-	-	+	-	+	-	+
Phlogacanthus thyrsiformis	+	+	-	-	-	+	-	-
Ziziphus mauritiana	-	-	-	-	+	+	-	+

(+) = Present (-) = Absent

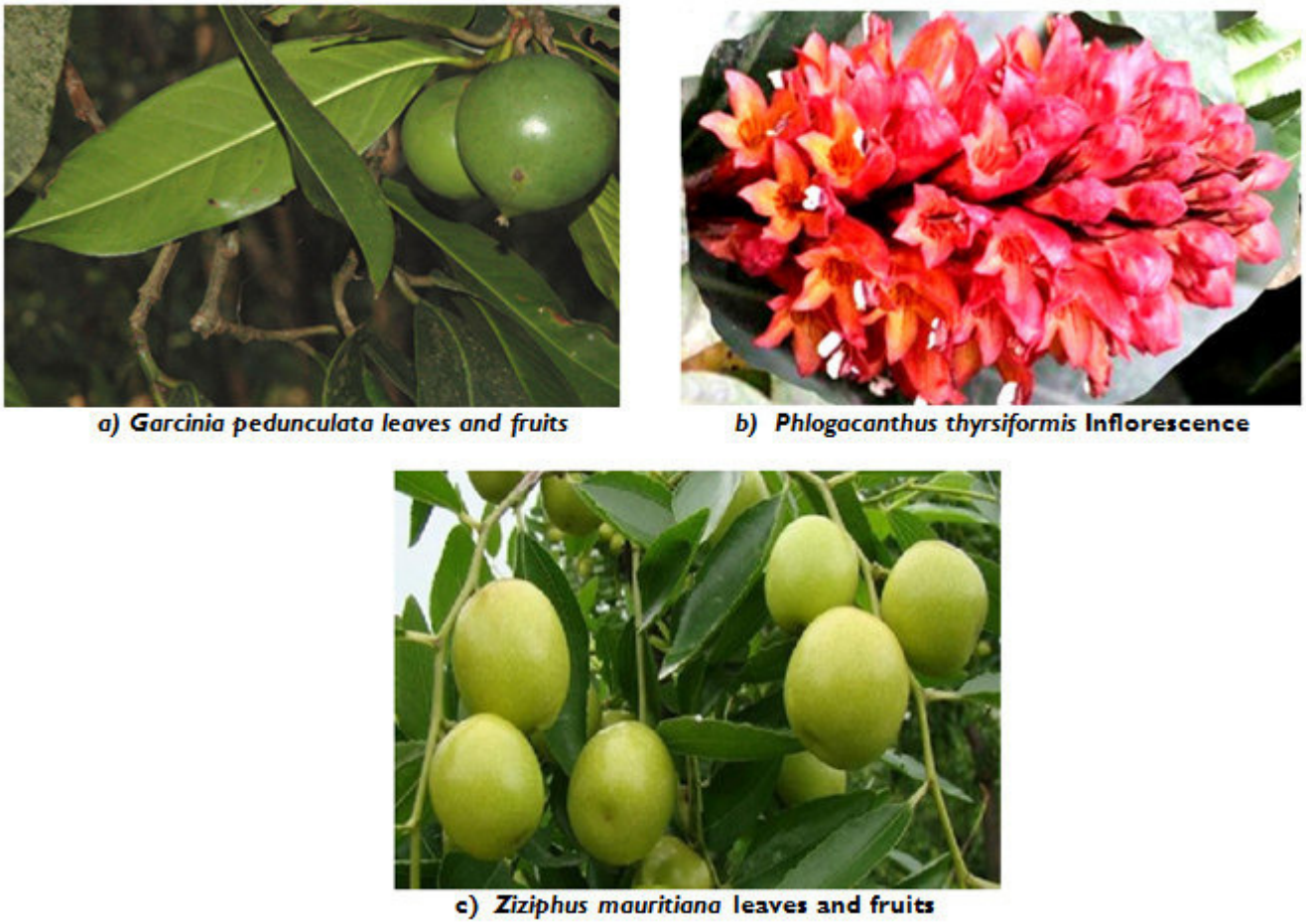
Table 4: MIC of the plant extracts against the bacterial pathogens

Plant extracts	MIC in (µg/mL)						
	E. coli	*S. aureus*	*P. vulgaris*	*K. pneumoniae*	*P. aeruginosa*	*B. cereus*	*S. marcescens*
Garcinia pedunculata	2560±0.00	2560±0.04	2560±0.00	2560±0.02	1280±0.00	1280±0.00	1280±0.03
Phlogacanthus thyrsiformis	1280±0.01	1280±0.01	1280±0.02	1280±0.01	1280±0.00	5120±0.02	1280±0.02
Ziziphus mauritiana	5120±0.02	5120±0.00	5120±0.03	5120±0.02	2560±0.02	2560±0.01	5120±0.03

Table 5: Plasmid curing by plant extracts

Strain (Plasmid)	Antibiotic resistance markers	Petroleum ether extract *Phlogacanthus thyrsiformis*		Chloroform extract *Ziziphus mauritiana*		Benzene extract *Garcinia pedunculata*	
		Sub-inhibitory conc. (µg/mL)	Efficiency of curing (%)	Sub-inhibitory conc. (µg/mL)	Efficiency of curing (%)	Sub-inhibitory conc. (µg/mL)	Efficiency of curing (%)
E. coli K12 (RP4)	Ap^r, Km^r, Tc^r	640±0.00	38±0.01	2560±0.03	62±0.01	1280±0.02	10±0.00
E. coli (pBR322)	Ap^r, Tc^r	640±0.03	42±0.03	1280±0.04	57±0.02	640±0.03	9±0.00
E. coli (pRK2013)	Ap^r, Km^r	640±0.01	35±0.02	2560±0.01	49±0.01	1280±0.01	12±0.00

Growth from SIC was serially diluted and plated onto Luria agar to get isolated colonies. 300 colonies were replica plated onto Luria agar and Luria agar containing antibiotic (10µg/mL). Colonies that grew on Luria agar but not on Luria agar containing antibiotics were considered as cured colonies.



a) *Garcinia pedunculata* leaves and fruits

b) *Phlogacanthus thyrsiformis* Inflorescence

c) *Ziziphus mauritiana* leaves and fruits

Fig 1: a) *Garcinia pedunculata* leaves b) *Phlogacanthus thyrsiformis* Inflorescence and c) *Ziziphus mauritiana* leaves used in dried form for the preparation of extracts

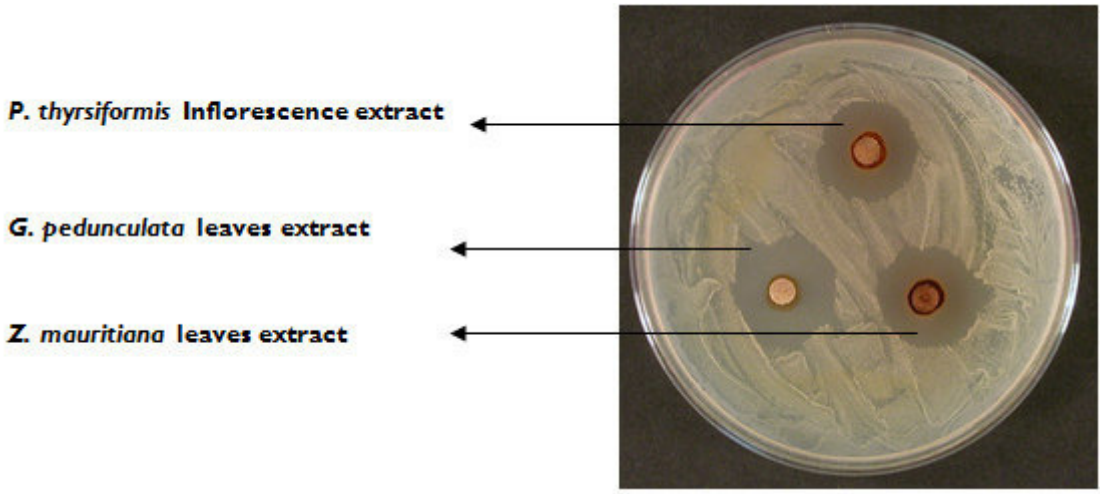


Fig 2: Antibacterial activity of *P. thyrsiformis* inflorescence extract, *G. pedunculata* and *Z. mauritiana* leaves extract against *Proteus vulgaris*

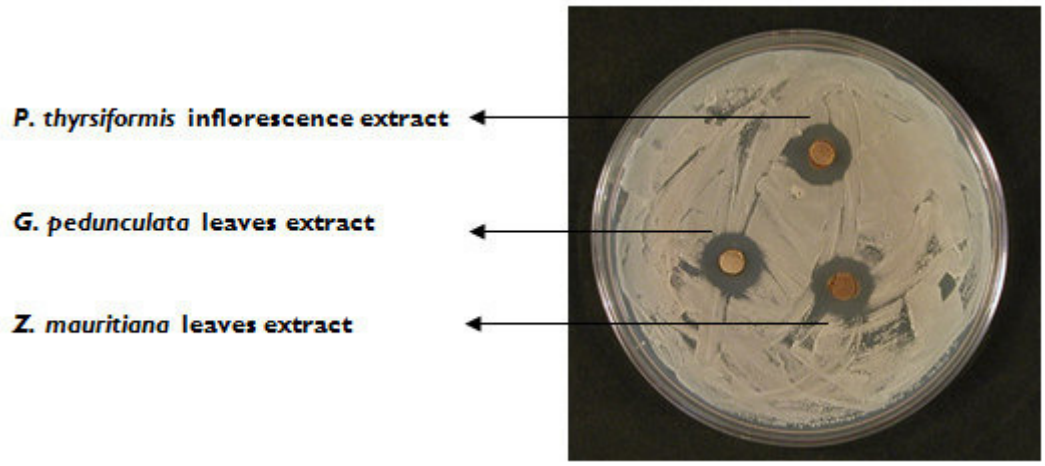


Fig 3: Antibacterial activity of *P. thyrsiformis* inflorescence extract, *G. pedunculata* and *Z. mauritiana* leaves extract against *E. coli*

Fig 4: Antifungal activity of *G. pedunculata* leaves extract against *Aspergillus flavus*

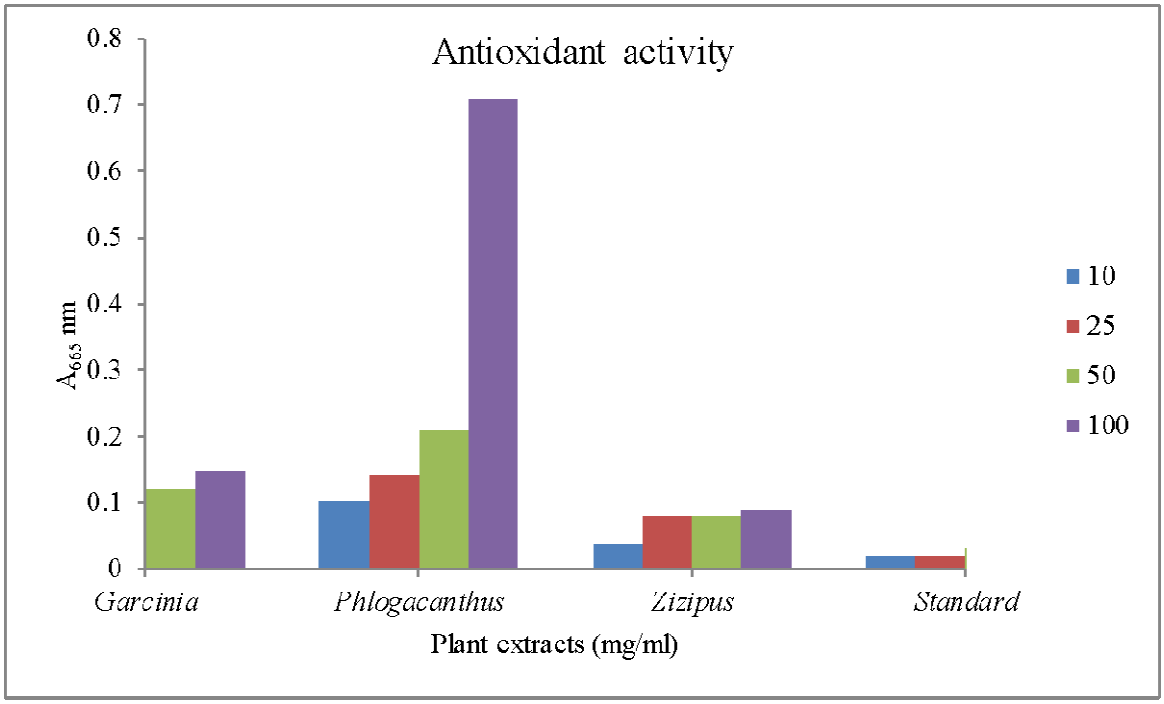


Fig 5: Antioxidant activity of the plant extracts viz., *Garcinia pedunculata*, *Phlogacanthus thyrsiformis and Ziziphus mauritiana* compared with standard (H_2O_2).

In the present investigation, *Phlogacanthus thyrsiformis* has shown greater antioxidant activity as compared to standard (H_2O_2) as well as *Garcinia pedunculata* and *Ziziphus mauritiana.* At 100 mg/ml concentration, *Phlogacanthus thyrsiformis* has shown maximum antioxidant activity.

4. CONCLUSION

The findings and the outcome of this research may be useful from the point of view of therapy of patients as the plant extracts of *Garcinia pedunculata*, *Phlogacanthus thyrsiformis* and *Ziziphus mauritiana* can serve as a potential source of antimicrobial and antioxidant agents which would be useful in controlling the growth of various pathogenic bacteria. The present investigation has discovered that extracts of plants of *Garcinia pedunculata, Phlogacanthus thyrsiformis* and *Ziziphus mauritiana* could be effectively used to remove the plasmid encoded antibiotic resistance. These results are of significance as plasmid encoded antibiotic resistance is a serious challenge for clinicians to treat. Ineffective antibiotics could become effective if plasmid encoded antibiotic resistance is removed from the population.

These plant extracts can cure plasmid encoded antibiotic resistance and make the pathogenic bacterial strains sensitive to the antibiotics. This can make the antibiotic treatment effective. This would be a novel approach towards controlling the multidrug resistant bacterial infections. Further research is essential towards isolation and identification of active compounds present in the extracts which could be used for pharmaceutical purposes.

5. AUTHOR CONTRIBUTION STATEMENT

The experimental work has been done by Aditi Jedhe and Vaishnavi Choudhary. Aparna Gunjal has planned, designed the experiments, and aided in manuscript writing. Rajashree Patwardhan, a corresponding author has guided in designing the experiments and has played a dominant role in manuscript writing.

6. CONFLICT OF INTEREST

Conflict of interest declared none.

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