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## Research Article

# *In Vitro* Evaluation of Antimicrobial Activity of Crude Extract from Plants *Diospyros peregrina, Coccinia grandis* and *Swietenia macrophylla*

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## Abstract

***Purpose:*** *The aim of the present study was to investigate antimicrobial activity of methanol extract of Diospyros peregrina fruits (MEDP), Coccinia grandis leaves (MECG) and Swietenia macrophylla barks (MESM).*
***Methods:*** *MEDP, MECG and MESM were examined against some selective gram positive and gram negative bacterial (20) and fungal (4) strains. Preliminary antimicrobial activity was evaluated by agar disc diffusion method. Minimum inhibitory concentration was determined by tube dilution (MIC) whilst minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined by agar diffusion method.*
***Results:*** *MEDP and MESM both have shown highest sensitivity against Escherichia coli strains. MEDP was found resistant to Sarcina luteus and Bacillus spp whereas MESM was resistant to all Shigella strains. MECG has shown major activity against Staphylococcus aureus, Escherichia coli, Shigella dysenteriae, Shigella soneii and Pseudomonas aeruginosa; whilst resistant to Shigella flexneri and Shigella boydii. Against fungi strains extracts were found effective at higher concentrations. Candida albicans has shown highest sensitivity whilst Penicillium spp. was least effective to all three extracts.*
***Conclusion:*** *The study confirms that MEDP, MECG, MESM all possess antimicrobial activity with different potency against variety of selected microorganisms. The differentiating activities of these three extracts encourage developing a novel broad spectrum antimicrobial herbal formulation in future.*

***Key words:*** *Diospyros peregrina, Coccinia grandis, Swietenia macrophylla, Antimicrobial activity, Ciprofloxacin, Griseofulvin.*

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## INTRODUCTION

In recent times, the rapid development of multi-resistant bacterial and fungal strains of clinically important pathogens fetches the interest of scientist to develop newer broad spectrum antimicrobial agents [1]. The less availability and high cost of new generation antibiotics necessitates looking for the substances from alternative medicines with claimed antimicrobial activity. A number of herbs with significant antimicrobial activity have been reported in different traditional literatures [2, 3, 4]. Now it is aimed to explore scientifically the antimicrobial potential of three traditional plants and substantiate the folklore claims.

*Diospyros peregrina* Gurke. (Ebenaceae) is a small middle sized tree of costal West Bengal. The fruits have ethnomedicinal significance for the treatment of diarrhoea, dysentery, cholera, ulcer of mouth and in wounds [5, 6]. The fruits contain triterpenes, alkanes, flavonoids and tannins [7, 8, 9, 10]. *Coccinia grandis* (L.) Voigt. (Family: Cucurbitaceae) is a climbing perennial herb distributed almost all over the world. The leaves of the plant possess antidiabetic, anti-inflammatory, antipyretic, analgesic, antispasmodic, antimicrobial, cathartic, expectorant activities [11, 12]. The leaves contain triterpenoids, alkaloids and tannins [13]. The plant *Swietenia macrophylla* (Family: Meliaceae) is a large evergreen tree native to tropical America distributed almost all over the world. The barks of this plant possess anti-HIV, antimicrobial, antimalarial, and antitumor activities [14]. The barks contain triterpenoids, limonoids, flavonoids and tannins [15, 16]. The objective of this research was to authenticate the antimicrobial sensitivity of the methanol extract of unripe matured fruits of *Diospyros peregrina, Coccinia grandis* leaves and *Swietenia macrophylla* bark and against some selected bacterial and fungal strains to lengthen the queue of antimicrobial herbs.

## MATERIAL AND METHOD

*Plant material*

Matured unripe fruits of *Diospyros peregrina* (Family: Ebenaceae) were collected in the month of June from the villages of South 24 Parganas, West-Bengal, India; the leaves of *Coccinia grandis* (L) Voigt. (Family: Cucurbitaceae) and barks of *Swietenia macrophylla* King. (Family: Meliaceae) were collected in the month of April, from the villages of Midnapore (E), West Bengal, India. The plants were authenticated by the Botanical Survey of India. Voucher specimens number entitled CHN/1-1(69), CNH/1-1 (44) and CNH/1-1(64) were deposited at our institute for future reference.

***Preparation of methanol extract***

The powdered plant materials (matured unripe fruits of *Diospyros peregrina,* leaves of *Coccinia grandis* and barks of *Swietenia macrophylla*) of 600 g each were extracted separately with methanol using Soxhlet apparatus. The resulting extracts were evaporated in vacuum and finally lyophilized into solid mass devoid of solvent (Yield = 8.75, 13.02 and 13.62 % respectively) and stored in desiccators for future use.

Preparation of sample

In the study of antimicrobial activity, extracts were dissolved in Dimethyl sulphoxide (DMSO). The corresponding concentration was expressed in term of µg of extract per ml of solvent (µg/ml).

***Chemicals***

All chemicals and solvents used in this experiment were of analytical grade obtained from BDH, Poole, UK.

*Microorganisms*

Twenty different bacterial strains namely *Staphylococcus aureus* 29737, *Staphylococcus aureus* ML 267, *Sarcina luteus* 9341*, Bacillus pumilus* 8241*, Bacillus subtilis* ATCC 6633*, Escherichia coli* ATCC 10536, *Escherichia coli* VC Sonawave 3:37 C*, Escherichia coli* CD/99/1*, Escherichia coli* $RP_4$*, Escherichia coli* 18/9, *Escherichia coli* K88, *Shigella dysenteriae* 1*, Shigella soneii* 1, *Shigella soneii* BCH 217, *Shigella flexneri* type 6, *Shigella boydii* 937, *Pseudomonas aeruginosa* ATCC 25619*, Vibrio cholerae* 2*, Vibrio cholerae* 785*, Vibrio cholerae* 1037 and four different fungal strains namely *Candida albicans* ATCC 10231, *Aspergillus niger* ATCC 6275, *Penicillium notatum* ATCC 11625, *Penicillium funiculosum* NCTC 287 were collected from institute of microbial technology, Chandigarh, India. The bacterial strains were

**Table 1:** Preliminary antimicrobial activity of MEDP, MECG and MESM

| | *Zone of inhibition diameters in mm* | | | |
|---|---|---|---|---|
| | **MEDP (200 µg/ml)** | **MECG (200 µg/ml)** | **MESM (200 µg/ml)** | **Ciprofloxacin (200 µg/ml)** |
| **Gram positive bacteria** | | | | |
| *Staphylococcus aureus* 29737 | 10.10 ± 0.26 | 12.56 ± 0.18 | 8.63 ± 0.12 | 14.13 ± 0.07 |
| *Staphylococcus aureus* ML 267 | 10.07 ± 0.20 | 13.20 ± 0.20 | 9.03 ± 0.17 | 13.53 ± 0.67 |
| *Sarcina luteus* 9341 | - | 10.00 ± 0.20 | 8.03 ± 0.13 | 12.63 ± 0.12 |
| *Bacillus pumilus* 8241 | - | 8.03 ± 0.12 | 8.07 ± 0.09 | 13.03 ± 0.12 |
| *Bacillus subtilis* ATCC 6633 | - | 8.00 ± 0.17 | 7.57 ± 0.03 | 13.60 ± 0.10 |
| **Gram negative bacteria** | | | | |
| *Escherichia coli* ATCC 10536 | 10.53 ± 0.13 | 12.53 ± 0.15 | 9.60 ± 0.10 | 13.50 ± 0.10 |
| *Escherichia coli* VC Sonawave3:37 C | 10.57 ± 0.09 | 12.57 ± 0.23 | 9.76 ± 0.03 | 13.00 ± 0.10 |
| *Escherichia coli* CD/99/1 | 12.20 ± 0.09 | 12.50 ± 0.20 | 10.13 ± 0.13 | 12.63 ± 0.70 |
| *Escherichia coli* $RP_4$ | 11.63 ± 0.03 | 12.00 ± 0.15 | 9.53 ± 0.90 | 12.13 ± 0.07 |
| *Escherichia coli* 18/9 | 12.50 ± 0.15 | 11.67 ± 0.13 | 10.30 ± 0.10 | 13.00 ± 0.12 |
| *Escherichia coli* K88 | 12.56 ± 0.09 | 11.60 ± 0.10 | 10.67 ± 0.07 | 14.06 ± 0.09 |
| *Shigella dysenteriae* 1 | 8.57 ± 0.13 | 13.03 ± 0.17 | - | 15.63 ± 0.07 |
| *Shigella soneii* 1 | 8.03 ± 0.13 | 13.07 ± 0.17 | - | 15.07 ± 0.13 |
| *Shigella soneii* BCH 217 | 8.50 ± 0.10 | 12.60 ± 0.15 | - | 15.57 ± 0.09 |
| *Shigella flexneri* type 6 | 8.13 ± 0.07 | - | - | 15.07 ± 0.12 |
| *Shigella boydii* 937 | 7.57 ± 0.03 | - | - | 14.43 ± 0.13 |
| *Pseudomonas aeruginosa* ATCC 25619 | 8.50 ± 0.10 | 14.10 ± 0.15 | 8.10 ± 0.12 | 16.07 ± 0.13 |
| *Vibrio cholerae* 2 | 10.00 ± 0.12 | 10.03 ± 0.12 | 8.63 ± 0.12 | 14.03 ± 0.13 |
| *Vibrio cholerae* 785 | 10.00 ± 0.21 | 10.43 ± 0.13 | 8.67 ± 0.13 | 14.60 ± 0.06 |
| *Vibrio cholerae* 1037 | 10.06 ± 0.03 | 11.63 ± 0.12 | 8.06 ± 0.12 | 14.07 ± 0.13 |
| **Fungal strains** | **MEDP (2000 µg/ml)** | **MECG (2000 µg/ml)** | **MESM (2000 µg/ml)** | **Griseofulvin (2000 µg/ml)** |
| *Candida albicans* ATCC 10231 | 10.70 ± 0.06 | 16.50 ± 0.15 | 11.20 ± 0.10 | 18.2 ± 0.20 |
| *Aspergillus niger* ATCC 6275 | 8.26 ± 0.12 | 11.97 ± 0.17 | 9.60 ± 0.10 | 14.03 ± 0.09 |
| *Penicillium notatum* ATCC 11625 | 8.60 ± 0.10 | 9.03 ± 0.17 | 8.53 ± 0.07 | 11.10 ± 0.10 |
| *Penicillium funiculosum* NCTC 287 | 7.33 ± 0.13 | 7.03 ± 0.09 | 7.63 ± 0.13 | 12.06 ± 0.06 |

**Key:** *'-' no measurable zone. Values are mean ± S.E.M. of 3 replications. MEDP – methanol extract of mature fruits of Diospyros peregrina, MECG – methanol extract of the leaves of Coccinia grandis, MESM – methanol extract of the bark of Swietenia macrophylla.*

grown in MacConkey agar plates at 37 $^0$ C and maintained on nutrient agar slants, while fungi were grown at 30 $^0$C and maintained in Saboraud glucose agar slants.

Preliminary screening for antimicrobial activity

The test was performed by disc diffusion assay as per NCCLS, 1993 [17]. The nutrient agar plates containing an inoculum size of $10^6$ cfu / ml for bacteria and 2 × $10^5$ spores for fungi on Saboraud glucose agar plates, were used [18]. Previously prepared extract impregnated disc (6 mm in diameter) at the concentrations of 200 µg/ml for bacterial and 2000 µg/ml for fungal strains were placed aseptically on sensitivity plates with appropriate controls. Ciprofloxacin (200 µg/ml) and griseofulvin (2000 µg/ml) were used as standard antibacterial and antifungal antibiotics respectively. Plates were incubated at 37 $^0$C for 24 hours for bacteria and 30 $^0$C for 3 days for fungal spores [19]. Sensitivity was recorded by measuring the clean zone of growth inhibition on agar surface around the disc.

**Table 2:** Minimum inhibitory concentration of the three methanol extracts

| Name of the organisms | *MEDP (µg/ml)* | | *MECG (µg/ml)* | | MESM (µg/ml) | |
|---|---|---|---|---|---|---|
| **Gram positive bacteria** *Staphylococcus aureus 29737* | MIC | MBC | MIC | MBC | MIC | MBC |
| | 100 | 100 | 10 | 25 | 200 | 200 |
| *Staphylococcus aureus* ML 267 | 100 | 150 | 10 | 25 | 200 | 200 |
| *Sarcina luteus* 9341 | > 200 | > 200 | 100 | 150 | 200 | 200 |
| *Bacillus pumilus* 8241 | > 200 | > 200 | 200 | > 200 | 200 | 200 |
| *Bacillus subtilis* ATCC 6633 | > 200 | > 200 | 200 | > 200 | 200 | 200 |
| **Gram negative bacteria** | | | | | | |
| *Escherichia coli* ATCC 10536 | 25 | 25 | 10 | 25 | 50 | 50 |
| *Escherichia coli* VC Sonawave 3:37 C | 25 | 25 | 10 | 25 | 50 | 50 |
| *Escherichia coli* CD/99/1 | 10 | 10 | 10 | 25 | 50 | 75 |
| *Escherichia coli* $RP_4$ | 10 | 10 | 10 | 25 | 50 | 75 |
| *Escherichia coli 18/9* | 10 | 10 | 25 | 50 | 50 | 75 |
| *Escherichiacoli* K88 | 10 | 25 | 25 | 25 | 50 | 75 |
| *Shigella dysenteriae* 1 | 200 | 200 | 10 | 10 | > 200 | > 200 |
| *Shigella soneii* 1 | 200 | 200 | 10 | 25 | > 200 | > 200 |
| *Shigella soneii* BCH 217 | 200 | 200 | 10 | 25 | > 200 | > 200 |
| *Shigella flexneri type* 6 | 200 | > 200 | > 200 | > 200 | > 200 | > 200 |
| *Shigella boydii* 937 | 200 | > 200 | > 200 | > 200 | > 200 | > 200 |
| *Pseudomonas aeruginosa* ATCC 25619 | 200 | 200 | 10 | 10 | 200 | > 200 |
| *Vibrio cholerae* 2 | 100 | 150 | 100 | 150 | 200 | 200 |
| *Vibrio cholerae* 785 | 100 | 150 | 100 | 150 | 200 | > 200 |
| *Vibrio cholerae* 1037 | 100 | 200 | 100 | 150 | 200 | > 200 |
| **Fungal strains** | | MFC | | MFC | | MFC |
| *Candida albicans* ATCC 10231 | 800 | 900 | 200 | 300 | 800 | 1000 |
| *Aspergillus niger* ATCC 6275 | 1500 | 1800 | 800 | 1000 | 1000 | 1200 |
| *Penicillium notatum* ATCC 11625 | 1500 | 1800 | 1500 | 1500 | 1500 | 2000 |
| *Penicillium funiculosum* NCTC 287 | 1500 | 2000 | 1500 | 2000 | 1500 | 2000 |

**Key:** *Mean values from three replicates are recorded, MIC – Minimum Inhibitory Concentration, MBC – Minimum Bactericidal Concentration, MFC – Minimum fungicidal Concentration*

Determination of Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC) and Minimum fungicidal Concentration (MFC) MIC was determined by tube dilution method for each of the test organism in triplicates [20]. To 0.5 ml of varying concentrations of the extracts (0 – 200 µg/ml for bacterial strains and 0 - 2000 µg/ml for fungal strains), 2ml of nutrient broth was added and then a loopful of test organism previously diluted to 0.5 McFarland turbidity standard for (Bacterial isolates) and $10^6$ cfu/ml (for fungal strains) was introduced to the tubes. The procedures were repeated on the test organisms using standard antibiotics ciprofloxacin (for bacteria) and griseofulvin (for fungi). A tube containing nutrient broth only seeded with the test organisms was served as control. Tubes containing bacterial cultures were then incubated at 37 $^0$C for 24 hours for bacteria and 30 $^0$C for 3 days for fungal spores. After incubation the tubes were

examined for microbial growth by observing the turbidity.

To determine the MBC and MFC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes which did not show any growth and inoculated on sterile nutrient agar (for bacteria) and Saboraud glucose agar (for fungi) by streaking. Plates inoculated with bacteria and fungi were then incubated at 37 $^0$C for 24 hours and 30 $^0$C for 3 days respectively. After incubation the concentration at which no visible growth was seen was noted as MBC (for bacteria) and MFC (For fungi).

## RESULTS

The *in vitro* antimicrobial activity of MEDP, MECG and MESM were shown in table 1. The MEDP and MESM have shown maximum zone of inhibition against *Escherichia coli* K88 of 12.56 and 10.67 mm respectively whilst MECG produced maximum zone diameter of 14.10 mm against *Pseudomonas aeruginosa* ATCC 25619. The activity of MEDP, MECG and MESM among fungi strains was found highest with *Candida albicans* ATCC 10231 (10.7, 16.5 and 11.2 mm respectively) and lowest with *Penicillium funiculosum* (7.33, 7.03 and 7.63 mm respectively). In this preliminary antimicrobial assay ciprofloxacin (200 µg/ml), griseofulvin (2000 µg/ml) were taken as standard antibacterial and antifungal agents. The results of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were shown in table 2. The results showed that MEDP is highly sensitive against *Escherichia coli* strains (MIC and MBC 10 - 25 µg/ml), moderately sensitive (MIC 100 µg/ml and MBC 100 - 150 µg/ml) to *Staphylococcus aureus* and *Vibrio cholerae* strains, less sensitive (MIC 200 µg/ml) to *Shigella* spp. and *Pseudomonas aeruginosa* whilst resistant (MIC and MBC > 200 µg/ml) to *Sarcina luteus* and *Bacillus spp*. MECG has shown maximum activity against gram-positive organism including *Staphylococcus aureus* (MIC 10 µg/ml and MBC 25 µg/ml) and gram negative cultures including *Escherichia coli* (MIC 10 - 25 µg/ml and MBC 25 - 50 µg/ml), *Shigella dysenteriae* (MIC and MBC 10 µg/ml), *Shigella soneii* (MIC 10 µg/ml and MBC 25 µg/ml) and *Pseudomonas aeruginosa* (MIC and MBC 10 µg/ml); moderately sensitive (MIC 100 µg/ml and MBC 150 µg/ml) to *Vibrio cholerae*, *Sarcina luteus*, less sensitive (MIC 200 µg/ml and MBC > 200 µg/ml) to *Bacillus* spp., whilst resistant (MIC and MBC > 200 µg/ml) to *Shigella flexneri* and *Shigella boydii.* MESM was found maximum sensitive (MIC 50 µg/ml and MBC 50 - 75 µg/ml) to *Escherichia coli* strains; less sensitive (MIC 200 µg/ml) to *Vibrio cholerae, Pseudomonas aeruginosa, Staphylococcus aureus, Sarcina luteus and Bacillus* spp. and resistant (MIC and MBC > 200 µg/ml) to all *Shigella* spp. Against fungi strains all extracts were found effective at higher concentrations. *Candida albicans* has shown highest sensitivity with MIC values of 800, 200, 800 µg/ml and MFC values of 900, 300, 1000 µg/ml with MEDP, MECG and MESM respectively whilst *Penicillium* spp. were found least effective with MIC and MFC values of 1500 µg/ml and 2000 µg/ml respectively with all three extracts.

## DISCUSSION

The antimicrobial activities of various plants have been reported by many researchers [21, 22]. Phytoconstituents present in plants namely flavonoids, alkaloids, tannins and triterpenoids are producing exciting opportunity for the expansion of modern chemotherapies against wide range of microorganisms [23, 24]. In present study a variety of gram positive, gram negative bacteria and fungal stains were selected for the screening of antimicrobial effect of three selected plant extracts to perceive the antimicrobial spectrum as well to authenticate ethnomedicinal claims. The results of this study showed that the MEDP, MECG and MESM have varied antimicrobial activities against the tested organisms. Among these three extracts MECG was found most effective against selected strains followed by MEDP and MESM in order effectiveness. Thus in search of novel broad spectrum antimicrobial agent, the formulation comprising different proportions of these extracts may be proven good. This study has not only shown the scientific basis for some of the therapeutic uses of traditional

plants, but also confirmed the ethnomedicinal claims for the selected plants.

## CONCLUSION

In conclusion, the results of this investigation revealed that methanol extracts of all three plants possess differentiating antimicrobial activity against selected bacterial and fungal strains. The differentiating activities against variety of microorganisms of these three extracts encourage developing a novel broad spectrum antimicrobial formulation in future. Now our research will be directed to develop a broad spectrum antimicrobial herbal formulation with these plants.

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