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COMPARATIVE EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF ETHANOL EXTRACT OF *TAXUS BACCATA, PHYLLANTHUS DEBILIS, PLECTRANTHUS AMBOINICUS* AGAINST MULTI DRUG RESISTANT BACTERIA

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ABSTRACT: Ethanol extract of the leaves of *Taxus baccata*, *Phyllanthus debilis*, *Plectranthus amboinicus* were studied for their antimicrobial property against multidrug resistant bacterial strains (MDR) from clinical specimens. Of these three plants, the most potent antimicrobial plant was *Phyllanthus debilis* followed by *Taxus baccata* (MIC range 80 - 400µg/ml) having broad spectrum activity, whereas *Plectranthus amboinicus* had no effect against MDR isolates. *Taxus baccata* and *Phyllanthus debilis* can be used as antimicrobial agents. Further confirmation needs ethano-pharmcological studies.

INTRODUCTION: The different systems of medicine practiced in India, Ayurveda, Siddha, Unani, Amchi and local health traditions, utilize a large number of plants for the treatment of human diseases [1].

It is estimated that 80% of the black population is consulting with traditional healers [2]. Medicinal plants are natural resources; yielding valuable herbal products which are often used in the treatment of various ailments [3].

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Antibiotics provide the main basis for the therapy of bacterial infections. Since the discovery of these antibiotics and their uses as chemotherapeutic agents there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases. However, overuse of antibiotics has become the major factor for the emergence and dissemination of multi-drug resistant strains of several groups of microorganisms [4].

In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world, the situation is alarming in developing as well as developed countries due to indiscriminate use of antibiotics. The drug-resistant bacterial pathogens have further complicated the treatment of infectious diseases in immune compromised, and cancer patients [5].

Multidrug resistant strains was a major nosocomial pathogen which causes severe morbidity and mortality worldwide. The evidence of rapid global spread of resistant clinical isolates, the need to find new antimicrobial agents is of paramount importance[4]. The aim of this study was to evaluate and compare the antimicrobial activity of *Taxus baccata, Phyllanthus debilis, Plectranthus amboinicus,* herbal extracts. The selection of these medicinal plants is based on their traditional uses in India [1]; information obtained from the siddha medicine literature [6] and reported antimicrobial activity of *Taxus baccata* [5], against ATCC strains.

MATERIALS AND METHODS:

Plant description: *Taxus baccata* is a Tree, belongs to the family Taxaceae, *Phyllanthus debilis* and *Plectranthus amboinicus* are herbs belongs to the family phyllanthaceae and Lamiaceae [6].

Plant material: The Dried leaves of three different plants viz.,*Taxus baccata, Phyllanthus debilis, Plectranthus amboinicus* were procured from ayurvedic shop and were authenticated by faculty from the Department of Botany, Mahatma Gandhi Memorial college Manipal, University of Mangalore.

Preparation of plant extract: The ethanol extract of leaves of *Taxus baccata, Phyllanthus debilis, Plectranthus amboinicus* was prepared by soxhlation [7]. The leaves were washed with distilled water and air-dried at room temperature for seven days. Then pulverized with clean mortar and pestle to fine powder and stored in a sterilized glass container at room temperature (25 - 30°C) until used. The powdered plant material (200 g) was repeatedly extracted in a 1000 mL round-bottomed flask with 500 mL ethanol (95%). The extraction was done until the solvent in the Soxhlet turned colourless (3days). The extracts were cooled at room temperature, filtered using Whatman no.1 filter paper. The ethanolic extract of the leaves was concentrated to dryness using water bath at 50^{o}C for 7 days and stored desiccated in refrigerator until further use [8].

Microbial Test strains: 20 bacterial strains isolated from urine and sputum samples were identified according to Cowan and Steel [9]. The bacterial strains identified were *Escherichia coli four strains, Klebsiella pneumonia* four strains, *Pseudomonas aeruginosa* two strains, *Acinetobacter baumannii* one strain, *Enterobacter cloacae* one strain, *Enterococcus faecalis* three strains, Methicillin Resistant *Staphylococcus aureus* (MRSA) three strains, *Providencia rettgeri* two strains. These strains were showed resistance to penicillins, first and second generation cephalosporins, Aminoglycosides, Fluroquinolones groups of antibiotics.

The anti-microbial spectrum of the following ATCC Strains [10] were also used Coagulase Positive *Staphylococcus aureus* (COPS) (ATCC25923), *Coagulase Negative Staphylococcus aureus* (CONS) (ATCC 35984), *Enterococcus faecalis* (ATCC 29212), *MRSA* (ATCC 43300), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27853), *Acinetobacter baumannii* (ATCC 19606), *Enterobacter cloacae* (ATCC 13847), *Proteus vulgaris* (ATCC 29905), *Salmonella typhi* (ATCC 14028).

Culture Medium and Inoculum: The MDR bacterial isolates were cultured on to nutrient agar (Hi-Media Pvt. Ltd., Bombay, India), freshly grown at 37°C were inoculated in peptone water to a McFarland turbidity standard of 0.5 (10^6 colony forming units (CFU) per ml) [11].

Antimicrobial Assay: The standard inoculums of test strains were introduced to Muller Hinton agar plates with a sterile glass spreader, by distributing the inoculums equally. Wells were punched in the agar plate with help of sterile borer. 50 µl plant material extract was added to the well with the help of sterile pipette. Plant material in two concentrations 8 mg and16 mg/50 µl were tried in duplicate [12]. Then all the plates were incubated at 37^{o}C for maximum 48 hours. Strains with inhibition zones were considered sensitive to the extract, those without such a zone were considered resistant.

Determination of Minimum Inhibitory Concentration: Agar dilution method was performed to calculate Minimum Inhibitory Concentration (MIC) values. Serial dilution of the extracts were added in Muller Hinton agar and poured onto petri plates, organism to be tested was inoculated and incubated at 37^{o}C.

Plates were examined for presence or absence of growth of bacteria after 48 hours. The highest dilution that yielded no bacterial growth on solid medium was taken as MIC [13].

RESULTS AND DISCUSSION: Emergence of multi-drug resistance in human and animal pathogenic bacteria as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drugs of plant origin [5].

In this study, the MDR strains and ATCC strains were sensitive to the antimicrobial activity of *Taxus baccata*, *Phyllanthus debilis*, whereas they exhibited strong resistance to the extracts of Plectranthus *amboinicus*. The most potent antimicrobial plant was *Phyllanthus debilis*(MIC range 80-300µg/ml)having broad spectrum activity, whereas *Taxusbaccata* showed antimicrobial effect against Gram positive MDR strains (MIC range 200-210 µg/ml) and exhibited higher MIC against Gram negative MDR strains. The mean MIC values of MDR strains and ATCC strains were shown in **Table 1 and 2** and comparative MIC values of different plant extracts are shown in **chart 1** respectively Drug-resistant strains of bacteria were found to be sensitive to the tested plant extracts. This has clearly indicated that antibiotic resistance does not interfere with the antimicrobial action of plant extracts and these extracts might have different modes of action on test organisms [5].

The antimicrobial activity of the *Taxus baccata* is may be due to presence of lignans [14] flavonoids [15]. The antimicrobial potency of other plants is also believed to be due to tannins, saponins, phenolic compounds, essential oils and flavonoids [1].

The antimicrobial property of *Taxus baccata* showed similar results to this study with varying degrees of potency. The difference inpotency may be due to the stage of collection of the plant sample, different sensitivity of the test strains and method of extraction [5]. Further studies are required to identify the compounds responsible for antimicrobial activity, the toxicity of the active constituents, their side effects, pharmaco-kinetic properties [5] and this can be used for future ethano-pharmacological studies and drug development.

TABLE 1: MIC VALUES OF MDR TEST STRAINS

MDR Isolates	*Taxus baccata* µg/ml	*Phyllanthus debilis* µg/ml	*Plectranthus amboinicus* µg/ml
Escherichia coli	360	150	>400
Enterobacter cloaecae	380	260	>400
Enterococcus faecalis	210	300	>400
Pseudomonas aeruginosa	350	180	>400
Providencia rettgeri	300	250	>400
Klebsiella pneumonia	290	150	>400
MRSA	200	80	>400
Acinetobacter baumannii	400	290	>400

TABLE 2: MIC VALUES OF ATCC STRAINS

ATCC STRAINS	*Taxus baccata* µg/ml	*Phyllanthus debilis* µg/ml	*Plectranthusam boinicus* µg/ml
CONS	20	10	>400
COPS	50	30	>400
Enterococcus faecalis	180	260	>400
Pseudomonas aeruginosa	300	100	>400
Enterobacter cloaecae	300	150	>400
Klebsiella pneumoniae	250	50	>400
Proteus vulgaris	200	80	>400
Salmonella typhi	150	60	>400
Escherichia coli	150	40	>400
MRSA	200	80	>400

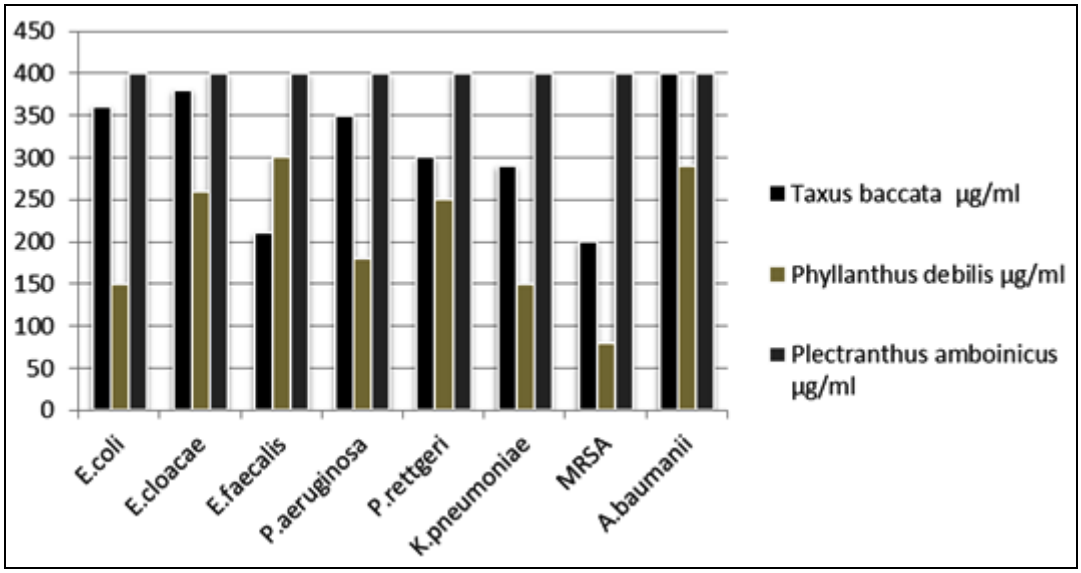

CHART 1: COMPARATIVE MIC VALUES OF *TAXUS BACCATA, PHYLLANTHUS DEBILIS* AND *PLECTRANTHUS AMBOINICUS*

CONCLUSION: *Phyllanthus debilis* has broad spectrum activity with low MIC values as compared to other two extracts *activity* but *Plectranthus amboinicus* had no antimicrobial activity against MDR strains.

The present study provides an important basis for the use of ethanol extract from the *Taxus baccata* and *Phyllanthus debilis* showed promising quantifiable antimicrobial activity against MDR bacteria causing health care acquired infections and can be used for future ethano-pharmacological studies and drug development.

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