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Original Research Article

PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF VARIOUS SOLVENT EXTRACTS OF *PUNICA GRANATUM* AND *PHYLLANTHUS NIRURI*

N. CHANDRASEKHARNATH, B. VENKANNA, Y.V. MAHLAKSHMI, A. UMA*

Center for innovative research, Institute of Science and Technology, JNTU-Hyderabad-500072

Author for Correspondence: uma.vedavathi@gmail.com

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ABSTRACT

The development of antimicrobial drugs or antibiotics is major challenge in the growing world because long term usage of commercially available drugs to treatment of various microbial diseases, after prolonged use of synthetic drugs may be pathogens get resistance from that particular synthetic drugs and also have their adverse effects, so alternative strategies are to develop the effective drugs from traditional medicine The aim of the present study to evaluate the antimicrobial activity of *Punica granatum* peels and whole plant of *phyllanthus niruri* extracts such as low polar to high polar solvent extracts. In folk medicine peels of *Punica granatum* and whole plant of *phyllanthus niruri* has been commonly used for treatment of various ailments like diarrhea, various infectious diseases. Aqueous, methanolic and ethanolic extracts of *Punica granatum* peels and whole plant of *phyllanthus niruri* show an average inhibitory zone diameter of 12,14,16mm respectively which indicates that ethanolic extract shows best result having ZOI greater than that of the standard antibiotics ampicillin is 14 mm. Methanolic extract of *Punica Granatum* and whole plant of *phyllanthus niruri* has lowest MIC of 1.45,1.36µg/ml showing that it is most effective as compared to MICs of other extracts.

Key words: *Punica Granatum*, *phyllanthus niruri*, Antibacterial activity, Minimum Inhibitory Concentration, Zone Of Inhibition.

INTRODUCTION

Traditional plant herbs and species are major source for animal food, have been used for many years in folk medicine and to enhance odor, flavour, and aroma of foods. In addition to boosting this activities herbs and species are also known their antioxidant, antifungal, antimicrobial and also used for treating various ailments (Shobana & Naidu 2000). In olden days there are many herbal medicines like curcumin, mahanimbamine, azadirectin are used for treatment for various microbial diseases ¹. Now a days there are many commercial drugs available in the market to treat various microbial diseases but they have side effects and also leads to serious complication like allergic response, nausea, vomiting, diahorea, wounds, gastrointestinal diseases, kidney stones and severe doses it leads to death. The alternative strategies are to develop the new drugs form natural source plants ⁹⁻¹⁰.

From the beginning of the last century, evidences on the antimicrobial activitites of have been accumulating. *Punica granatum* peels and whole plant of *phyllanthus niruri* medicinally used for treatment of various microbial diseases because they contain various phytochemicals like polyphenols, alkaloids, terpinoids, tannins but there are no scientific reports for their scientific validation⁴.

The main aim of the present work was to examine the efficacy of different solvent extracts of the major phytochemical

compounds from *Punica granatum* peels ⁸ and whole plant of *phyllanthus niruri* traditionally used for their medicinal properties and evaluate the preliminary phytochemical analysis and their antimicrobial activities ³.

MATERIALS AND METHODS

Collection of plant material and extraction

The fresh *Punica granatum* peels and whole plant of *phyllanthus niruri* was collected from vishakapatnam hill ranges of vishakapatnam district, Andharapradesh, India. *Punica granatum* peels and *Phyllanthuss niruri* whole plant were dried and shade and pulverized to a course powder and extracted with no polar (hexane) to high polar solvent (water) using the soxhlet apparatus. The filtrate was obtained and remove to solvent dryness at 50-60 C in a rotaevaporator too obtain dark molten mass.

Phytochemical analysis

The procedures described by Harbone with few changes were used to analyse the phychochemical compounds in the extracts ²⁰.

Test for steroids:

10 ml of the plant extract was evaporated to dry mass and dissolved in 0.5 ml of solvent. Acetic anhydride (0.5 ml) and 2ml of concentrated sulphuric acid were added. A green color or blue color or a mixture of these two colors was indicated as positive for in the presence of steroid compounds.

Test for Tannins:

1 cm³ of freshly prepared 10% KOH was added to 1 cm³ of the extract. A dirty white precipitate indicated the presence of tannins. Powdered coarse powder of test plant (1.0) was weighed into a beaker and 10 ml of distilled water added. The mixture was boiled for five minutes. Two drops of 5% FeCl₃ were then added. Production of greenish precipitate indicated the presence of tannins.

Test for Flavanoids:

A small piece of magnesium ribbon was added to extract of the plant material, this was followed by the drop wise addition of concentrated hydrochloric acid. Colors varying from orange to red crimson to magenta indicated flavonones.

Test for Alkaloids:

The extract of plant sample (0.5g) was stirred with 5ml of HCL on a steam bath. The solution obtained filtrate was treated with two drops of Mayer's reagent. The two solutions were mixed and made up to 100 ml with distilled water. Turbidity of the extract filtrate on addition of Mayer's reagent was regarded as evidence for the presence of alkaloids.

Test for saponins:

The extract of plant sample (0.5gr) was introduced in to a tube containing 5.0 ml of distilled water and shake Vigorously for 2 minutes formation of froth indicates the presence of saponins.

Test for glycosides:

The extract of plant sample 1gr added in to separate beakers .to one of the beakers was added 5ml dilute sulphuric acid while 5ml

sulphuric acid is added to other beaker. The two beakers were heated for 3-5 minutes and the contents Filtered in to labeled test tubes. The filtrate was made alkaline with 5% sodium hydroxide and heated with Fehling's Solution for 3 mins. The presence of reddish precipitate in the acid filtrate and the absence of such precipitate in the aqueous filtrate were regarded as positive for glycosides.

ANTIMICROBIAL ACTIVITY**Test organisms for antimicrobial activity**

In order to determine the antibacterial activity of different solvent extracts of *Punica granatum* peels and whole plant of *phyllanthus niruri*, different gram negative and gram positive bacteria were used *Klebsiella pneumonia*, *Enterococci*, *Escherichia coli*(15), *Pseudomonas aeruginosa*, *Staphylococcus aureus* were obtained of Institute of Microbiology (IMTECH), Chandigarh and Department of Biotechnology, Jawaharlal Nehru Technological University, Hyderabad. The strains were maintained and tested on nutrient agar for antimicrobial tests ⁵.

Antimicrobial activity

The agar disc diffusion method was employed for the antibacterial activity of different solvent plant extracts. The discs (6 mm diameter) impregnated with different concentrations of extracts placed on the surface of the petridishes containing 20 ml of nutrient agar media for bacterial strains for antimicrobial activity seeded with 100µl of microbial cultures. (5X10⁶ CFU/ml). The plates were incubated for 24 hr at 35±2°C for bacteria. The zone of inhibition formed

around the discs were measured and expressed in millimeters. All the experiments were done at triplicate ⁶.

Determination of the minimal inhibition concentration

The minimal inhibitory concentration (MIC) values, which is represent the lowest plant extracts concentrations that completely inhibits the growth of microorganisms, were determined by a micro-well dilution method in a 96 well micro titer plates. From previously prepared different microbial suspension cultures (10^5 CFU/ml) was added to each well plates were incubated for 18 hr at 37°C and then were examined with Elisa reader at 620 nm and the lowest concentration of each extract showing no growth was takes as its minimum inhibitory concentration (MIC). All the samples tested in triplicate ¹¹.

RESULTS AND DISCUSSION

Phytochemical analysis

In the preliminary phytochemical analysis of the peels of *Punica granatum* and whole plant of *phyllanthus niruri* was showing encouraging results for the presence of various secondary metabolic compounds like alkaloids, flavanoids, polyphenols, tannins, glycosides and steroids. Etanolic and methanolic extracts contains rich flavanoids, tannins and alkaloids, water extract contain glycosides and hexane extract contain very few bioactive compounds ¹⁴.

Antimicrobial activity

Nearly 80% of the world populations depends on the traditional medicine for primary health care, mainly including the

use of natural products . Researchers have extensively studied the biological properties of *Punica granatum* and whole plant of *phyllanthus niruri* extracts their results showed that this plant is ethno medically valuable ¹⁶ . *Punica granatum* peel and whole plant of *phyllanthus niruri* extracts arecurrently used for treatment of UTI diseases and in the preparation of therapeutic formulae(18-19). The tannin rich ellagitannins and phenolic acids of *Punica granatum* have antibacterial, antifungal and antiprotozoal activity. In the current study the hot aqueous, methanolic and ethanolic extracts of *Punica granatum* and whole plant of *phyllanthus niruri* extracts showed Zone of inhibition of atleast 12mm against *P.aeruginosa* which was greater than that of ampicillin 14 , 16mm against *E.coli* which was a little lesser than that of Standard (16mm) and 14mm against *E.coli* which was greater than thatof standard ampicillin (16mm) respectively. The antibacterial activity of peels of *Punica granatum* and whole plant of *phyllanthus niruri* extracts may beindicative of presence of metabolic toxins or broad spectrum antimicrobial compounds that act against both gram +ve and gram -ve bacteria. Ethanolic extracts exhibited higher degree of antibacterial activity as compared tothat of other extracts tested against bacteria that cause gut infection, stomachache, diarrhea ¹⁷. Reported that *P.granatum* and whole plant of *phyllanthus niruri* contains large amount of alkaloids (25%) and antibacterial activity may be

indicative of presence of secondary metabolites. The ethanolic extract of *P. granatum* and whole plant of *phyllanthus niruri* extracts showed some extent of antibacterial activity against *E.coli* and *S. aureus*¹²⁻¹³.

CONCLUSION

In the present study an attempt has been made to decipher the preliminary screening of plant extracts and antimicrobial activity of peels of *Punica granatum* and whole plant of *phyllanthus niruri* extracts (which are generally treated as wastes). Peels of *Punica granatum* and whole plant of *phyllanthus niruri* extracts are reported to have polyphenols, flavonoids, alkaloids, tannins, and glycosides other polyphenols as bioactive compounds in previous studies. All the extracts have antibacterial activity against both gram positive and gram negative bacterial strains. After further purification and characterization of the active metabolites present in *Punica granatum* and whole plant of *phyllanthus niruri* extracts followed by detailed study of various biological activity and pharmacological effects.

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Table.1. phytochemical screening of different extracts of peel of *Punica granatum* and whole plant of *phyllanthus niruri*

S.NO	Secondary metabolites	Hexane	Ethylacetate	Methanol	Ethanol	Water
1	Flavonoids	-	-	++	++	++
2	Alkaloids	+	+	+++	+++	+
3	Tannins	+	++	+++	+++	+
4	Glycosides	-	+	++	++	+++
5	Steroids	++	++	+	+	-
6	Saponins	-	++	+++	+++	++

Table.2. In vitro antibacterial activity of different extracts of peel of *Punica granatum* and whole plant of *phyllanthus niruri*

Test organisms	ZONE OF INIHIPTIONS (mm)								
	Aqueous extracts			Methanolic extracts			Ethanollic extracts		
	PG*	PN*	AA*	PG*	PN*	AA*	PG*	PN*	AA*
<i>Klebsiella</i>	12	11	11	13	12	12	12	12	10
<i>Enterococci</i>	13	14	10	14	16	10	13	13	11
<i>E.coli</i>	16	12	12	16	13	11	14	14	12
<i>Pseudomonas</i>	14	13	10	12	14	10	16	13	13
<i>S.aureus</i>	12	15	11	13	11	11	12	13	11

PG*: Peel of punica granatum

PN*: Whole plant of *phyllanthus niruri*

AA*: Ampicillin Antibiotic