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GC–MS ANALYSIS, *IN VITRO* ANTI-OXIDANT AND ANTI-MICROBIAL ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *PUNICA GRANATUM* (L) ROOT(S)

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ABSTRACT

The present study was aimed to investigate anti-oxidant and anti-microbial activities of root(s) of *Punica granatum* hydroalcoholic extract in DPPH method and cup plate and disc diffusion method respectively. The extract was GC-MS for the confirmation of active phytoingredients responsible for the desired biological response. Both the studies showed the presence of various compounds like quercetin, polyphenols, propanethiol etc. The anti-microbial activity done by cup plate and disc diffusion method showed broad spectrum antimicrobial activity by acting on both gram positive and gram negative pathogens, which was compared with ciprofloxacin.

Key Words: *Punica granatum*, hydroalcoholic extract, anti-microbial activity

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INTRODUCTION

Throughout the ages humans have relied on Nature to cater for their basic needs, not the least of which are medicines for the treatment of a wide spectrum of diseases. Plants, in particular, have formed the basis of sophisticated traditional medicine systems, with the earliest records, dating from around 2600 BCE, documenting the uses of approximately 1000 plant-derived substances in Mesopotamia. These include oils of *Cedrus* species (cedar) and *Cupressus sempevirens* (cypress), *Glycyrrhiza glabra* (liquorice), *Commiphora* species (myrrh), and *Papaver somniferum* (poppy juice), all of which are still used today for the treatment of ailments ranging from coughs and colds to parasitic infections and inflammation. Egyptian medicine dates from about 2900 BCE, but the best known record is the "Ebers Papyrus" dating from 1500 BCE, documenting over 700 drugs, mostly of plant origin. The Chinese Materia Medica has been extensively documented over the centuries, with the first record dating from about 1100 BCE (Wu Shi Er Bing Fang, containing 52 prescriptions), followed by works such as the Shennong Herbal (~100 BCE; 365 drugs) and the Tang Herbal (659 CE; 850 drugs). Likewise, documentation of the Indian Ayurvedic system dates from before 1000 BCE (Charaka; Sushruta and Samhitas with 341 and 516 drugs respectively). The Greeks and Romans contributed substantially to the rational development of the use of herbal drugs in the ancient Western world. Dioscorides, a Greek physician (100 CE), accurately recorded the collection, storage, and use of medicinal herbs during his travels with Roman armies throughout the then "known world", whilst Galen (130–200 CE.), a practitioner and teacher of pharmacy and medicine in Rome, is well known for his complex prescriptions and formulae used in compounding drugs. The Arabs,

however, preserved much of the Greco-Roman expertise during the Dark and Middle Ages (5th to 12th centuries), and expanded it to include the use of their own resources, together with Chinese and Indian herbs unknown to the Greco-Roman world. A comprehensive review of the history of medicine may be found on the website of the National Library of Medicine (NLM), United States National Institutes of Health (NIH). Plant-based systems continue to play an essential role in healthcare, and their use by different cultures has been extensively documented. The World Health Organization (WHO) estimated in 1985 that approximately 65% of the population of the world predominately relied on plant-derived traditional medicines for their primary health care, while plant products also play an important, though more indirect role in the health care systems of the remaining population who mainly reside in developed countries. A survey of plant-derived pure compounds used as drugs in countries hosting WHO-Traditional Medicine Centers indicated that, of 122 compounds identified, 80% were used for the same or related ethnomedical purposes and were derived from only 94 plant species. Some relevant examples are khellin, from *Ammi visnaga* (L) Lamk., which led to the development of chromolyn (in the form of sodium chromoglycate) as a bronchodilator; galegine, from *Galega officinalis L*., whichwas the model for the synthesis of metformin and other bisguanidine-type antidiabetic drugs; and papaverine from *Papaver somniferum* which formed the basis for verapamil used in the treatment of hypertension. The latter plant is better known as being the source of painkillers such as morphine and codeine, but probably the best example of ethnomedicine's role in guiding drug discovery and development is that of the antimalarial drugs, particularly quinine and artemisinin Malaria remains one of the greatest health challenges confronting humankind, and the search for better drugs, both in terms of efficacy and cost, is a global health imperative (1-4). The relationship between nature and human beings is as old as the dawn of humankind; the interest of people to nature has increased steadily along with the knowledge based on the experiences of the traditional healers and practitioners because humankind could not find any alternative source in the search for nutrition, poison or remedy as exclusive as nature. Although many treatment strategies and synthetic drugs with different characteristics had been designed and discovered along with developing technology and scientific innovations, the current reports about the treatment and drug preferences of patients and physicians displayed that there is a significant trust in natural products as complementary and alternative medicine and it is thought that patients and physicians give credit to the natural products because of the undesirable and sometimes unpredictable activities of the synthetic antibiotics or chemotherapeutic agents such as non-selective tissue damage , systemic toxicities , drug resistance or potential long-term side effects . However, it should be noted that natural plants and herbs are not precise, though they seem to be healthful.

MATERIALS AND METHODS

Collection of Plant Materials

Fresh roots of the plant *Punica granatum* were collected from the local regions of Andhra Pradesh. The roots were washed with tap water and shade dried. After shade drying, the roots were coarsely powdered and the powder was subjected to various studies for which the materials and methods presented below.

GC-MS Analysis of the Crude Extract

GC-MS analysis of these extract was performed using a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with an Elite-I, fused silica capillary column (30mmX0.25mm 1D X 1µMdf, composed of 100% Dimethyl polysiloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1ml/min and an injection volume of 2µl was employed (split ratio of 10:1); Injector temperature 250°C; Ion source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5seconds and fragments from 45 to 450 Da.

Total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbomass9. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The Name, Molecular weight and structure of the components of the test materials were ascertained.

Determination of DPPH Free Radicals Scavenging Activity (5)

Preparation of sample

10mg of hydroalcoholic extract of *Punica granatum* root(s) was dissolved in the 1ml of ethanol. Further, the serial dilution was carried out with the concentration of 5mg, 3mg, 1mg, 0.5mg, 0.25mg, 0.125mg respectively.

Preparation of DPPH

2mg of DPPH powder was dissolved in the 5ml of ethanol. Furthermore, 1ml was taken from the above mixture and dissolved in the 4ml of ethanol.

The absorbance was measured at 517nm against the corresponding blank solution which was prepared by taking 200µl of DPPH and 50µl of ethanol. The assay was performed in triplicates. Percentage inhibition of free radical DPPH was calculated based on blank reading by following equation.

Percentage of antioxidant = (A blank - A sample/A blank) X 100

Evaluation of Antibacterial Activity

Determination of zone of inhibition by cup plate method (6)

The cylinder plate assay of drug potency is based on measurement of the diameter of zone of inhibition of microbial growth surrounding cylinders (cups), containing various dilutions of test compounds. A sterile borer was used to prepare four cups of 6 mm diameter in the agar medium spread with the micro-organisms and 0.1 ml of inoculum. These cups were spread on the agar plate by spread plate technique. Accurately measured (0.05 ml) solution of each concentration and reference standards were added to the cups with a micropipette. All the plates were kept in a refrigerator at 2 to 8 °C for a period of 2 hours for effective diffusion of test compounds and standards. Later, they were incubated at 37°C for 24 hours. The presence of definite zone of inhibition of any size around the cup indicated antibacterial activity. The solvent control was run simultaneously to assess the activity of dimethylsulphoxide and water which were used as a vehicle. The experiments were performed three times. The diameter of the zone of inhibition was measured and recorded.

RESULTS AND DISCUSSION

GC-MS Analysis of Hydroalcoholic Extract of *Punica Granatum (L*) Root(S)

GC-MS analysis of the extract showed the presence of various phytocompunds in the hydroalcoholic crude extract. Retention time, % peak area, name of the phytocompound, molecular formula, molecular weight and the chemical structures of the various compounds were obtained and presented in the Table-1. Totally 11 number of compounds were obtained in the GC-MS analysis, which were responsible for the expected biological activity. This confirms the various secondary metabolites present in the roots of the *Punica granatum(L*) plant and its therapeutic uses.

Table-1 GC-MS Analysis of Hydroalcoholic Extract of *Punica granatum (l*) Root(s)

	Retention Time	Peak area %	Name of the Compound	Molecular formula	Molecular weight
	2.576	4.29	1-Propanethiol	C_3H_8S	76
	2.810	5.50	Ethane,1,1,1-triethoxy-O-acetic acid	$C_8H_{18}O_3$	162
	4.887	6.76	1,1,3-Triethoxybutane	$C_{10}H_{22}O_3$	190

	7.696	17.41	2-Isopropylpyrrolidine	$C_7H_{15}N$	113
	11.000	21.68	9-Aza-1-methylbicyclo[3.3.1]nonan-3-one	$C_9H_{15}NO$	153
	23.595	10.29	n-Heptadecanol-1	$C_{17}H_{36}O$	256
	24.069	9.31	Lidocaine	$C_{14}H_{22}N_2O$	234
	31.533	3.69	Xylitol, 1-O-octanoyl-	$C_{13}H_{26}O_6$	278
	31.683	3.51	5-O-Methyl-d-gluconic acid dimethylamide	$C_9H_{19}NO_6$	237
10.	32.546	6.17	Glyceroltricaprylate	$C_{27}H_{50}O_6$	470
11.	35.089	11.39	1,2-Benzenedicarboxylic Acid	$C_{24}H_{38}O_4$	390

The results of the study showed the hydroalcoholic extract of *Punica granatum(L*) roots can be used easily accessible source of natural anti-oxidant and as a possible food supplement or in pharmaceutical industry (Fig-1)

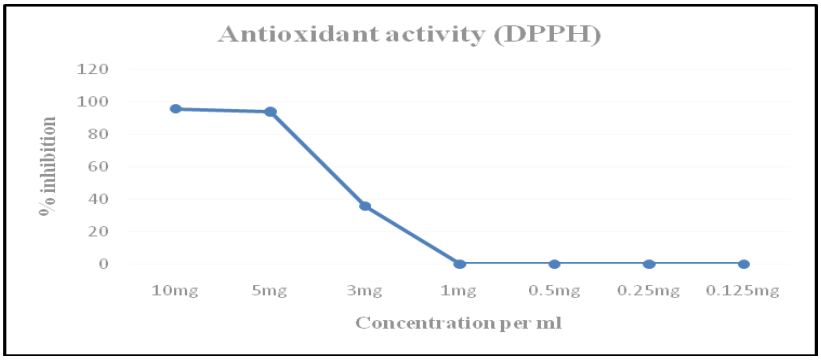

Fig-1 *Invitro* Antioxidant Activity of hydroalcoholic extract of *Punica granatum (l*) root(s) by DPPH free radicals Scavenging Activity

The results of the study showed the hydro alcoholic extract of *Punica granatum(L*) roots exhibited broad spectrum activity against the various gram positive and gram negative micro organisms. For instance, Ciproflaxacin showed the maximum zone of inhibition (27mm) against *Staphylococcus* sp. *Klebsiella pneumonia* (9mm), *Escherchia coli* (10mm), *Bacillus subtilis* (13mm) (Table-2)

Table-2 *Invitro* Antibacterial Activity of Hydroalcoholic Extract of *Punica granatum (l*) Root(s)

S.No	**Concentration (µg/ml)**		**Name of the Organisms**	**Zone of Inhibition (mm)**	
	Test	**Standard Ciprofloxacin**		**Test**	**Standard Ciprofloxacin**
1	50	5	*Klebsiella pneumonia*	17	9
2	50	5	*Escherchia coli*	8	10
3	50	5	*Bacillus subtilis*	11	13
4	50	5	*Staphylococcus aureus*	19	27
5	100	5	*Staphylococcus aureus*	21	

CONCLUSION

Punica granatum (L) root(s) commonly known as pomegranate is a member of the monogeneric family, Punicaceae and is found throughout the world. Its phytocompounds possess numerous biological and toxicological properties including anti-oxidant, anti-inflammatory, anti-cancer and anti-angiogenesis activities. The extract was GC-MS for the confirmation of active phytoingredients responsible for the desired biological response. Both the studies showed the presence of various compounds like quercetin, polyphenols, propanethiol etc. The anti-microbial activity done by cup plate and disc diffusion method showed broad spectrum antimicrobial activity by acting on both gram positive and gram negative pathogens, which was compared with ciprofloxacin.

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