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Research Article

ANTIBACTERIAL ACTIVITY OF PUNICA GRANATUM AND VITIS VINIFERA AGAINST URINARY TRACT INFECTION

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Abstract

Natural inhibitors for pathogenic microorganisms have been explored in many plants. Herbal medicine is the foundation for about 75-80% of the world population. Plant have an amazing ability to produce a wide variety of secondary metabolites, like alkaloids, glucose prepare the methanol, ethanol and acetone extracts of *Punica granatum* and *Vitis vinifera*. Determine the antimicrobial activity of Punica granatum and Vitis vinifera by using agar ditch diffused method. To analyse the phytochemical constituents of the promising plant extract.

Keywords: Antibacterial, *Punica granatum*, *Vitis vinifera*, UTI pathogen, Pytochemical.

INTRODUCTION

In recent years, the infectious diseases remain the leading cause of death worldwide and infections due to antibiotic resistant ability of some microorganisms. However, synthetic antimicrobial agents provide broad spectrum characteristics, but often associated with the adverse effects on the host, including immune suppression, hypersensitivity and several allergic responses (Selvakumar, 2015; Ahmad *et al*., 1998 and Yusuf *et al*., 2012). However, there is a drastic increase in the usage of herbal medicine was found in last few years from the developed countries (Vethanarayanan *et al*.,2011). Plants have an amazing ability to produce a wide variety of secondary metabolites, like alkaloids, glucose, terpenes, saponins, steroids, flavonoids, tannins, quinones and coumarin. These biomolecules are the source of plant derived antimicrobial substances (PDAms). Some natural products are highly efficient in the treatment of bacterial infections. Rising antibiotic resistance and the scarcity of new antimicrobials long been acknowledged. A major challenge in global health care is the need for novel, effective and affordable medicines to treat microbial infections, especially in developing countries of the world, where up to one half of deaths are due to infectious diseases. The problem of antibiotic resistance in humans and animals will continue for a long time. Against this backdrop the development of alternative drug classes to treat such infectious diseases is urgently required. Antimicrobial agents are essentially important in reducing the global burden of infectious diseases. According to the world health organization (WHO), medicinal plants would be the best source to obtain a variety of drugs. A urinary tract infection (UTI) is an infection in any part of your urinary system (kidneys, ureters, bladder and urethra). Most UTIs are caused by bacteria that enter the urethra and then the bladder. The infection most commonly develops in the bladder, but can spread to the kidneys. Most of the time, your body can get rid of these bacteria. However, certain conditions increase the risk of having UTIs.

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Women are at greater risk of developing a UTI than are men. Infection limited to your bladder can be painful and annoying. However, serious consequences can occur if a UTIs spreads to your kidneys. *Punica granatum* (pomegranate) is the small tree which measures between five and eight meters tall and mainly found in Iran, the Himalayas in northern India, China, USA and throughout the Mediterranean region (facial s.cornucopica: a source book of edible plants, 1990). Pg is one of the important endemic plants of Iran, growing in most regions throughout the country, in arid and semi arid regions due to its ability to adapt to adverse ecological conditions. Over 764 cultivars of *Punica granatum* (pg) have been collected during a germ plasm and grown in the cities of saveh and yazd(Iran), all of which posses specific fruit characteristics including size, colour, taste, time of ripening, and disease resistance (Sheridan *et al.,* 2007). The *Punica granatum* can be also divided into several anatomical compartments including seed, juice, peel, flower, bark, and root with each possessing interesting pharmacological and toxicological activities (Stover *et al*., 2007). The fruit of the *Punica granatum* has extensively been used as a traditional remedy against acidosis, dysentery, microbial infections, diarrhoea, helminth infection, haemorrhage and respiratory pathologies. *Punica granatum* seed have also been shown to contain the estrogenic compounds, estrone and estradiol (Kim and Choi, 2009). Furthermore, the dried pericarp and the juice of the fruit are considered beneficial for treatment of colic, colitis, menorrhagia, oxyuriasis, headache, diuretic, acne, piles, allergic dermatitis, and treatment of oral diseases. Recent studies have shown new scientific investigations for the traditional uses of *Punica granatum* (Ricci *et al*., 2006). Grapes (*Vitis vinifera* L.) Is one among the important and leading fruit crops grown worldwide. About 80%of the harvest is used in industries for wine making. The abundant phenolic compounds from grape seed are catechins, epicatechin, procaryanidin, and some dimmers and trimers. The polyphenols of grape seeds have been recognized for their beneficial role in human health. The grape seed is shown to exhibit bioactivities such as antioxidants, anti-inflammatory, anti-bacterial, anti-cancer, cardio protective, hepatoprotective, neuroprotective, antiaging and anti-diabetic. The oil extracted

firm grape seed is used in cosmetics, culinary, pharmaceutical and medicinal purpose.

MATERIALS AND METHODS

Selection of medicinal plant

In the present work, *Punica granatum* and *Vitis vinifera* plants were screened for potential antibacterial activity

Collection, identification and processing of fruit seed of *Punica granatum* and *Vitis vinifera*

Fresh plants materials were collected from Chidambaram, Cuddalore district, Tamilnadu. The taxonomic identifies of these plants were determined. The fruit of *Punica granatum* and *Vitis vinifera* were washed with 70% alcohol and then rinsed with sterilized distilled water. The juice of Punica granatum and *Vitis vinifera* were prepared by crushing the seeds aseptically by mortar and pestle and the seeds are dried for 10-12 days and powdered using mechanical grinder and then stored in air tight containers for further use.

Preparation of crude seed extracts

The powdered materials used for the preparation of methanol, ethanol, and acetone extracts.

Preparation of methanol extract: 10 grams of seed powder of Punica granatum and *Vitis vinifera* were soaked in 100ml of methanol in conical flask separately, plugged with cotton and kept at room temperature for 3 days and filtered through what man no:1 filter paper. The filtrate was evaporated in Petri dish at room temperature for 2-3 days till the volume was reduced to one-fourth of the original volume if the solvent used and stored at 4°c in airtight bottles.

Preparation of ethanol extract: 10 grams or seed powder of Punica granatum and *Vitis vinifera* were soaked in of ethanol in conical flask separately, plugged with cotton and kept at room temperature for 3 days and filtered through Whatman No:1 filter paper. The filtrate was evaporated in Petri dish at room temperature for 2-3 days till the volume was reduced to one- fourth of the original volume of the solvent used and stored at 4°c in airtight bottles

Preparation of acetone extract: 10 grams of seed powder of Punica granatum and *Vitis vinifera* were 100 cetone in conical flask separately, plugged with cotton and kept at room temperature for 3days and filtered through Whatman No:1 filter paper. The filtrate was evaporated in petri dish at room temperature for 2-3 days till the volume was reduced to one-fourth of the volume of the solvent used and stored at 4°c in airtight bottles to dissolve the extracts.

Bacterial strain used for assay

The bacterial cultures used in the study were procured from the department of microbiology, Annamalai University, Tamilnadu. The organisms Used were *staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli Enterococcus sp, Streptococcus pyogens, Klebsiella pneumonia, Proteus sp.* The strains were maintained in nutrient agar slant at 4°c and sub cultured on nutrient agar slant and incubated at 37°c for 24 hours before doing antimicrobial susceptibility test.

Agar diffusion method

The inoculation of microbes was prepared from bacterial culture. About 15-20 ml of Muller Hinton agar medium was poured in sterilized petri dishes and allowed to solidify. One drop of bacterial strain was spread over the medium by swab plate method. Sterile discs were impregnated with the different concentration of solvent extracts of *Vitis vinifera* 25µl, 40 µl and 50 µl and Punica granatum like 25µl,40 µl and 50 µl .The discs were allowed to dry and placed on the agar surface. The plates were incubated at 37°c for 24 hours. The antibacterial activities were evaluated by measuring inhibition zone diameters at the end of the incubation period.

Phytochemical qualitative analysis

The plant extracts and metabolic and ethanolic aqueous solutions were assessed for the existence of the phytochemical analysis by using the following standard methods.

Test for Anthraquinones: 10 ml of benzene was added in 6 g of ephedra powder sample in a conical flask and soaked for 10 minutes and then filtered. Further 10 ml of 10% ammonia solution was added to the filtrate and shaken vigorously for 30 seconds and pink, violet, or red colour indicated the presence of Anthraquinones in the ammonia phase.

Test for tannins: 10 ml of bromine water was added to the 0.5 g aqueous extraction. Declaration of bromine water showed the presence of tannins.

Test for saponins: 5.0 ml of the distilled water was mixed with the aqueous crude plant extract in a test tube and it was mixed vigorously. The frothing was mixed with few drops of olive oil and mixed vigorously and the foam appearance showed the presence of saponins.

Tests for flavonoids: Shinoda test: Pieces of magnesium ribbon and Hcl concentrated were mixed with aqueous crude plant extract after few minutes and pink colour showed the presence of flavonoids. Alkaline reagent test: 2ml if 2.0%NaOH mixture was mixed with aqueous plant crude extract; concentrated yellow colour was produced, which became colourless when we added 2 drops of diluted acid to mixture. This result showed the presence of flavonoids.

Tests for Glycosides: Lieberman's Test: We added 2.0 ml of acetic acid and 2 ml of chloroform with whole aqueous plant crude extract. The mixture was then cooled and we added H2SO 4 . Green colour showed the entity of aglycone, steroidal part of glycosides. Keller-kiliani test: A solution of glacial acetic acid (4.0ml) with 1 drop of 2.0% FeCl3 mixture with the 10 ml aqueous plant extract and 1ml H2SO 4. A brown rings formed between the layers which showed the entity of cardiac steroidal glycosides. Salkowski's Test: We added 2ml of H2SO 4 to the whole aqueous plant crude extract. A reddish brown colour formed which indicated the presence of steroidal aglycone part of the glycoside.

Test for terpenoids: 2.0 ml of chloroform was added with the 5ml aqueous plant extract and evaporated on the water path and then boiled with 3 ml of H2SO 4 concentrated. A grey colour formed which showed the entity of terpenoids.

Test for steroids: 2ml of chloroform and concentrated H2SO 4 were added with the 5ml aqueous plant crude extract. In the lower chloroform layer red colour appeared that indicated the presence of steroids.

RESULTS

The bacterial cultures viz., *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli Enterococcus sp, Streptococcus pneumonia and Proteus sp* procured from the department of microbiology, Annamalai university were tested against the ethanol, methanol and acetone extracts of Vitis vinifera and Punica granatum of different concentration like 25µl,40 µl and 50 µl concentrations.

Antibacterial activity of *Vitis vinifera*against UTI pathogens

Methanolic extract of *Vitis vinifera* showed maximum antibacterial activity against all the pathogens viz., Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, and Proteus sp than the ethanol and acetone extracts except staphylococcus aureus. Among all the organisms, maximum antibacterial activity was exhibited against the organism in the order *Proteus vulgaris, Staphylococcus aureus* and *Klebsiella pneumoniae*, however, ethanol extract of vitis vinifera showed maximum antibacterial activity against *Staphylococcus aureus* than the other pathogens.

Antibacterial activity of *Punica granatum* against UTI pathogens

Methanolic extract of *Punica granatum* showed maximum antibacterial activity against all the pathogens. The methanolic extract of Punica granatum showed maximum zone of inhibition (12±0.3) against *Proteus vulgaris* followed by *Klebsiella pneumoniae* (11±0.3) in the concentration of 50µl. The ethanolic and acetone extract showed highest inhibition against *Staphylococcus aureus* followed by *Proteus vulgaris*.

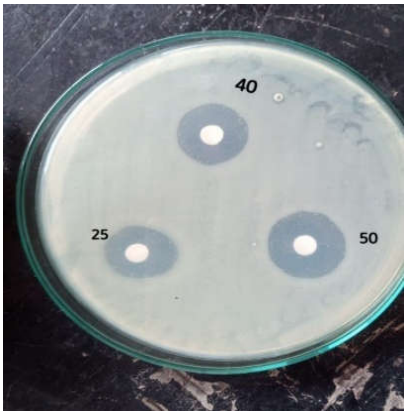

Figure A. Antimicrobial efficacy of *Punicagranatum* seed extracts against UTI causing pathogens

Figure B. Antimicrobial efficacy of *Vitisvinifera* seed extracts against UTI causing pathogens

Phytochemical Analysis of *Vitis Vinifera* Extract

The phytochemical screening was done with the methanolic seed extract of *Vitis vinifera*. The results revealed that *Vitis vinifera*has the several efficient secondary metabolites as phytochemicals. Table 3 showed the results of phytochemical analysis.

Table 1. Antibacterial activity of *Vitis vinifer a*against UTI pathogens

S.No.	Pathogen	Zone of Inhibition								
		Methanol			Ethanol			Acetone		
		25µl	40µl	50µl	25µl	40µl	50µl	25µl	40µl	50µl
1	*Staphylococcus aureus*	9±0.2	8±0.1	11±0.1	8±0.1	11±0.1	13±0.3	7±0.1	9±0.3	10±0.2
2	*Pseudomonas aeruginosa*	9±0.2	11±0.2	12±0.2	8±0.2	11±0.3	11±0.2	NZ	6±0.1	8±0.2
3	*Escherichia coli*	6±0.1	8±0.1	9±0.3	NZ	5±0.2	6±0.2	NZ	7±0.2	7±0.2
4	*Proteus vulgaris*	12±0.1	14±0.2	14±0.2	7±0.2	10±0.1	12±0.2	9±0.3	12±0.3	12±0.3
5	*Streptococcus pyogens*	6±0.2	6±0.3	8±0.1	NZ	6±0.1	8±0.3	NZ	6±0.3	7±0.1
6	*Klebsiella pneumoniae*,	8±0.3	11±0.2	11±0.2	8±0.2	10±0.2	12±0.3	8±0.2	9±0.1	11±0.2
7	*Enterococcus sp*	7±0.1	8±0.1	8±0.3	6±0.2	7±0.1	8±0.3	NZ	6±0.1	8±0.3

NZ=NO ZONE±SD

Table 2. Antibacterial activity of *Punica granatum*a gainst UTI pathogens

S. No.	Pathogen	Zone of inhibition								
		Methanol			Ethanol			Acetone		
		25µl	40µl	50µl	25µl	40µl	50µl	25µl	40µl	50µl
1	*Staphylococcus aureus*	8±0.1	10±0.2	11±0.2	8±0.2	10±0.1	12±0.2	8±0.2	9±0.1	11±0.3
2	*Pseudomonas aeruginosa*	5±0.2	9±0.1	9±01	NZ	6±0.2	8±0.1	NZ	6±0.2	8±0.2
3	*Escherichia coli*	NZ	5±0.1	8±0.1	NZ	6±0.3	7±0.2	NZ	7±0.2	6±0.3
4	*Proteus vulgaris*	8±0.1	11±0.2	12±0.3	9±0.3	12±0.2	11±0.2	8±0.2	11±0.1	11±0.2
5	*Streptococcus pyogens*	NZ	7±0.1	8±0.3	NZ	6±0.2	9±0.1	NZ	5±0.3	7±0.2
6	*Klebsiella pneumoniae*	8±0.2	8±0.2	11±0.1	8±0.3	11±0.1	11±0.2	8±0.2	9±0.2	10±0.3
7	*Enterococcus sp*	6±0.1	8±0.4	8±0.1	5±0.2	7±0.1	8±0.1	NZ	6±0.1	7±0.2

NZ=NO ZONE±SD

Table 3. Phytochemical analysis of *vitis vinifera* extract

S.No	Plant constituent	Methanolic extract
1.	Alkaloids	+++
2.	Cardiac glycosides	+
3.	Flavonoids	+++
4.	Glycosides	+++
5.	Phenols	+++
6.	Resins	+
7.	Saponins	+++
8.	Steroids	+++
9.	Tannins	+++
10.	Terpenoids	+++
11.	Triterpenoids	+

+++: highly present, ++: moderately present,
+: low,-:absent s:seed extract.

DISCUSSION

The resistance to multiple drugs has become a common feature in which most of the organisms associated with diarrhoea and other enteric diseases, urinary tract infection (Rahman *et al.,*1997) and wound infections (Uraih,2004) . In the present work the seed extracts of *Punica granatum* and *Vitis vinifera* were evaluated for the antimicrobial activity against certain urinary tract infections causing pathogens. The methanolic seed extracts of *vitis vinifera* exhibited higher antibacterial activity than the *Punica granatum*. According to Dwvedi and Gopal, (2010) the antibacterial activity described here in validate the ethnic information of curative use of *vitis vinifera* seed in inflammation and skin diseases. Additionally *v.vinifera* is being an edible plant even, its bark is used traditionally to treat diabetes suggesting absence of host toxicity a fact corroborated by this work. The antimicrobial effects of Punica granatum were previously studied. Indeed, it is reported that seed of *Punica granatum* are widely used as antimicrobial agent. The study also showed that ,all the extracts of the same plant possesses strong antibacterial activities against on both selected gram-positive and gram-negative bacteria, and similarly it was also reported (Palmer *et al.,* 2012; Gunsolley, 2010) that it had the ability to inhibit the activity of drug resistant *S.aureus* and *E.coli.* However it was in contradictory to the study that the water extracts of the *Punica granatum* didn't show any antibacterial activity against the standard and drug resistant *E.coli* strain in our investigation. Medina (2005) reported that methanol extracts of *Punica granatum* showed antifungal activity against *C.albicans*. These results are in accordance to results obtained in the present study for *C.albicans* where in antifungal activity was observed for all the four different solvent extracts.. These secondary metabolites are reported to have many biological and therapeutic properties, so this species is expected to have many medicinal uses. The extraction yield calculated for acetone methanol and ethanol extracts of parts of *v.vinifera* showed that methanol extract registered higher percentage of yield. It may be due to high polarity of methanolic solvent which can draw high variety of plant constituents than the other solvents did. However, flavonoids and saponins were rich in ethyl acetate extracts. It is explained that the polarity level and species nature are playing major role in extracting the secondary metabolites (Uraih,2004).

Conclusion

In the present work, *Punica granatum* and *vitis vinifera* seed extracts were screened for potential antibacterial activity.The urinary tract infection causing pathogens were procured from the department of microbiology, RMMCH, Annamalai University Chidambaram. Three different extracts viz methanol, ethanol, and acetone were prepared for *vitis vinifera* and *Punica granatum* seeds. The antibacterial efficacy of seed extracts of *vitis vinifera* and *Punica granatum* were tested by disc diffusion method. Among the three different tested extracts, methanol extract exhibited maximum zone of inhibition towards all the tested pathogens. Methanolic extract of *vitis vinifera* showed maximum zone of inhibition (14±0.2) against *Proteus vulgaris*, followed by zone of inhibition (12±0.2) against *Pseudomonas aeruginosa*. The methanolic extract of *vitis vinifera* was subjected to phytochemical screening. The results revealed that the seed extracts of *Vitis vinifera* had several phytochemicals such as alkaloids, flavonoids, glycosides, phenols, saponins.It is concluded that the seed extract of *Vitis vinifera* can be used for UTI infections after clinical trials.

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