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Evaluation Of Antioxidant And Antimicrobial Activities In Foliar Tissue Of Selected Medicinal Plants

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Abstract: The present investigation is aimed at investigating the in vitro antioxidant, lipid peroxidation and antimicrobial activities of selected medicinal plants viz. *Andrographis paniculata, Azima tetracantha, Pongamia pinnata, Punicia granatum* and *Ruellia patula.* The antioxidant screening was done by DPPH free radical scavenging assay using aqueous and ethanolic extracts. Significant antioxidant activity of ethanolic extracts and the aqueous extract was recorded in *Punicia granatum.* The antimicrobial activity of the plant extracts tested against *E.coli*, *Staphyloccoccus aureus* and *Klebsiella pneumoniae* also showed broad spectrum of antimicrobial activity in *Punicia granatum* but in other samples antimicrobial activity was observed at higher concentrations. Overall the results suggest that *Punicia granatum* is a potent source of natural antioxidant and antimicrobial agents.
Keywords: Medicinal plants, antioxidants, antimicrobial, lipid-peroxidation.

Introduction

Plants have been an essential part of human life since ancient times. Recently there is an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing free radical induced tissue damage[1]. In many parts of the world medicinal plants are still against bacterial, viral and fungal infections. India is endowed with a rich wealth of medicinal plants which in turn are a source of genetic diversity[2]. India is also a major exporter of raw MAPs and processed plant based drugs. Exports of crude drugs from India in 1994-95 were valued at US$ 53,219 million and of essential oils US$ 13,2500 million.

The growing interest in the investigation of antioxidants and antimicrobial agents from plants are due to two reasons. First, there is epidemical and clinical evidence suggesting that consumption of vegetables and fruits reduce the risk of developing chronic diseases (eg. Cancer) and secondly, phytochemicals are safer than synthetic chemicals[3]. Screening of various plant extracts for antioxidants, lipid peroxidation and antimicrobials activity were reported[4,5,6].

Because of the side effects and the resistance that the pathogenic microorganisms build against antibiotics, recent studies are focused on extracts and biologically active compounds isolated from plant species used in herbal medicine[7]. Plant based antimicrobials represent a vast untapped source of medicine and there is a need for exploration of antimicrobials in different plants.

Antimicrobials of plant origin have enormous therapeutic potential as they are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials[8]. Published reprints suggest that many of the antioxidant activities reported in plants might be due to phenolic compounds[9]. Flavonoids are a group of polyphenolic compounds with known bioactive properties, which include free radical scavenging inhibition of hydrolytic oxidative enzymes and anti-inflammatory action[10].

The present study aims at determining free radical scavenging activities of some of the commonly used medicinal plants in Tamil Nadu. The antioxidant activity of these plant extracts against lipid peroxidation, free radical scavenging activity against 2,2-diphenyl-1picrylhydrazyl (DPPH) and the antimicrobial activity were studied followed by rapid assays for various classes of phytochemicals.

Materials and Methods

Plant Material:

Medicinal plants used in the present study were collected from southern part of Tirunelveli district, Tamil Nadu, India, and the collected materials were authenticated by Dr.C.Livingstone, Head of the Department of Botany, Madras Christian College, Chennai. Voucher specimens were deposited herbarium of the institute.

Preparation of Extracts:

Healthy plants were collected, leaves were shade dried and powdered. Ten gm of the respective powder was mixed separately with 100ml of distilled water and 100 ml of ethanol for aqueous and ethanol extraction respectively. These mixtures were incubated for 24 hours with occasional shaking and filtered with Whatman filter paper to obtain filtrate which was further evaporated to obtain the extract. For the experiment to be performed stock solutions of aqueous and ethanol extracts 1mg/ml stock solution was prepared.

$$\% \text{ inhibition} = \frac{A_0 - A_{10}}{A_0} * 100$$

Where A_0 = absorbance at 0 min; A_{10} = absorbance at 10 min.

Lipid Peroxidation Inhibition Assay:

The extent of Lipid peroxidation in the presence or absences of the various extracts were determined in sheep liver homogenate in terms of TBARS by the method of Ohkawa *et al.*[13]. Sheep liver brought from the slaughterhouse of Tambaram, Chennai, Tamil Nadu was washed with ice cold Tris Hcl buffer (20 mM, pH 7.0); KCl (30 mM); FeSO4.7H2O (0.06 mM) and homogenized (25% w/v) in the same buffer with mortar and pestle. Homogenate was centrifuged at 4000 rpm for 5 minutes and the supernatant was used for this study.

The reaction mixture contained 0.1 ml of sheep liver homogenate, various concentrations of both aqueous and ethanolic extracts in the final volume of 0.5 ml of 8.1% sodium dodecyl sulphate (SDS), 1.5 ml of 20% acetic acid and 1.5 ml of 0.8 % aqueous solution of thiobarbituric acid (TBA). The pH of 20% acetic acid was adjusted with 1 N NaOH to 3.5. The mixture was finally made up to 4.0 ml with distilled water and heated at 95˚C for 60 min. After cooling under tap water, 1.0 ml of distilled water and 5.0 ml of n-butanol and pyridine (15:1 v/v) was mixed and the mixture was shaken vigorously on a vortex mixer. After centrifugation at 4000 rpm for 5 minutes the absorbance of the organic layer (upper layer) was measured

Phytochemical Screening of the Extracts:

Phytochemical screening was performed using standard procedures as described by Sofowora[11] and Trease & Evans[12] for the presence of various classes of compounds like Phenol, Alkaloid, Flavanoid, Tanin, Couramins, Sterols, Amino acids and Proteins.

DPPH Free Radical Scavenging Assay:

The free radical scavenging activities of the plant extracts against 2, 2 Diphenyl-1- Picryl Hydrazyl reduced were determined. Varying concentrations of the herbal extract were prepared from the stock solution. The reaction consisted of 1ml of 0.1mM DPPH in ethanol, 1ml of 0.05M tris HCl buffer, 1ml ethanol and 0.5ml of herbal extract. The tubes were kept in the dark and the absorbance was measured at 520nm. The decrease in absorbance at 520 nm was continuously recorded in a spectrophotometer for 10 min. the scavenging effect, expressed as the decrease of absorbance at 520 nm was observed and the percentage of DPPH radical-scavenging ability (SA) of the various extracts was calculated using the following formula.

immediately at 532 nm using appropriate controls in a spectrophotometer. Percent inhibition in lipid peroxidation was calculated by the following expression:

Percent inhibition = {(Ao-A_1)/ Ao} x 100

Where Ao is absorbance of control and A_1 is absorbance of sample.

Screening for Antimicrobial Activity:

The antimicrobial activities of the crude aqueous and ethanolic extracts of the plant samples were evaluated by antibiotic well assay method[14]. The solidified LB agar plates were inoculated with 100 µl of *E.coli* (MTCC-443), *Klebsiella pneumonia* (MTCC-109) and *Staphylococcus aureus* (MTCC-96) by swabbing. The wells were prepared on the agar plate already seeded with cultures (10^6 cfu/ml) with the help of a cork borer (10mm dia.). The wells were loaded with 50, 100,150 and 200 µg/ml volume of the extracts and 50 µl of ampicillin was used as a control. The plates were incubated at 37° C overnight. For each extract three replicate trials were conducted against each organism.

Table.1 Phytochemical constituents of selected medicinal plants.

Reaction	*A.paniculata*	*A.tetracantha*	*P. pinnata*	*P.granatum*	*R.patula*
Phenol	-	-	-	+	
Alkaloid	+	-	-	+	-
Flavanoid	+	+	+	+++	+
Tanin	-	+	-	+++	+++
Couramins	+	+	+	+	+
Sterols	-	-	-	-	-
Amino acids	+	+	+	+	+
Proteins	+	+	+	+	+

Number of + sign indicate the degree of response and – sign indicates no response

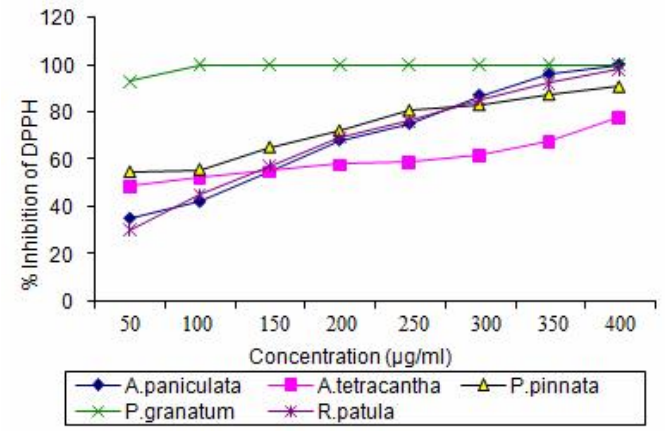

Figure.1 Inhibition of DPPH by the aqueous extracts of selected medicinal plants

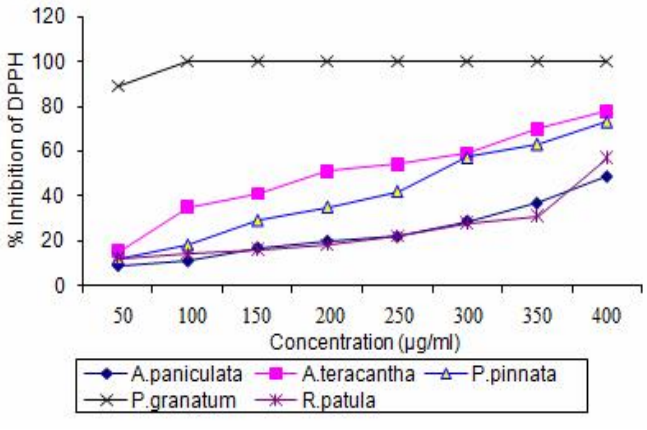

Figure.2 Inhibition of DPPH by the ethanolic extracts of selected medicinal plants

Results

Phytochemical Screening of the Extracts:

Phytochemical screening of all the plants tested revealed the presence of flavanoids, coumarins, amino acids and proteins (Table.1). *Punicia granatum* and *A.paniculata* responded positive to alkaloid. Phenolics substances were also recorded in *Punicia granatum*.

DPPH Free Radical Scavenging Assay:

All the evaluated samples showed moderate to significant free radical scavenging activities, the aqueous (Fig.1) and ethanolic extracts (Fig.2) of *Punicia granatum* showed higher (100%) free radical inhibition at 100 µg/ml concentration, while in other species maximum inhibition was observed at around 400 µg/ml concentration. As such, *Punicia granatum* extract was comparable to Ascorbic acid (Vitamin C), where 100% inhibition was observed at 7 µg/ml concentration. Comparative analysis indicates that *Punicia granatum* extract about 10 times less potent than the standard (ascorbic acid).

In-vitro lipid peroxidation inhibition Assay:

The effect of plant medicinal extracts and ascorbic acid standard on the *in-vitro* inhibition of lipid peroxidation is presented in Fig.3. The generation of lipid peroxides by $FeSO_4$ in sheep liver homogenate is inhibited by various plant extracts. The maximum inhibition of lipid peroxidation was found to be 39% for 250 µg/ml in the presence of *Pongamia pinnata* extract. The relative lipid peroxidation inhibition value for ascorbic acid (control) was found to be 69% at 250 µg/ml. Inhibition of lipid peroxidation was observed in other plant extracts was less significant. Many species of plants belong to this category[15].

Screening for Antimicrobial Activity:

Antimicrobial activity was observed in aqueous and ethanolic extracts of all the plants against *E.coli*, *K.pneumoniae* and *S.areus* at varying concentrations. The aqueous extracts of *P.granatum* showed antimicrobial activity against all the 3 bacterial species at 150 µg/ml. Aqueous extract of *A.paniculata* and the ethanolic extract of *A.tetracantha* inhibited *K.pneumoniae* and *S.areus* at 150 µg/ml. Ethanolic extract of *R.patula* exhibited antimicrobial activity against *E.coli* and *S.areus* at 150 µg/ml. Aqueous extracts of *A.tetracantha* and R.patula exhibited antimicrobial activity against *K.pneumoniae* at 150 µg/ml (Table.2). *T*he antimicrobial activity of ampicillin standard showed inhibitory effect on E.coli, K.pneumoniae and S.aureus at 50 µg/ml. In all other extracts, antimicrobial activity was observed at higher concentrations.

Table.2 Antimicrobial activity of selected medicinal plants figures indicate zone of inhibition in mm.

Extracts		*E.coli*	*K.pneumoniae*	*S.aureus*
Ampicillin		16	14	14
A.paniculata	Aqueous	-	16	12
	Ethanolic	-	-	-
A.tetracantha	Aqueous	-	12	-
	Ethanolic	-	14	16
P.pinnata	Aqueous	-	-	-
	Ethanolic	-	-	-
P.granatum	Aqueous	16	18	15
	Ethanolic	-	-	14
R.patula	Aqueous	-	14	-
	Ethanolic	15	-	13

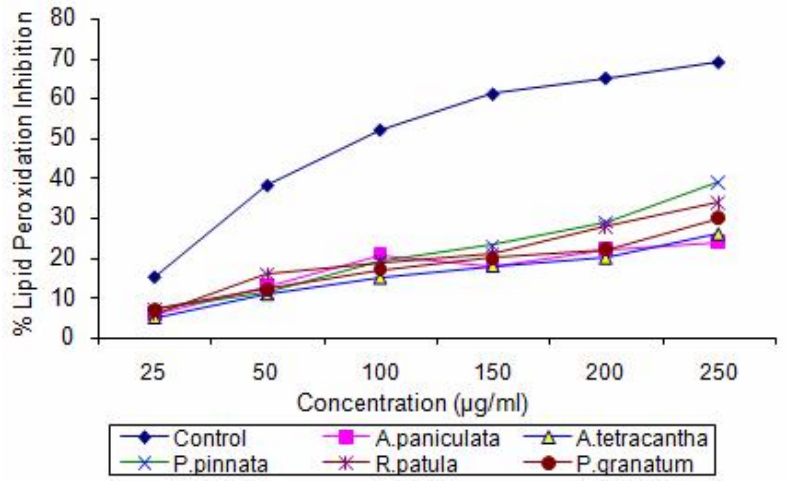

Figure3. Effect of Aqueous extract of various Medicinal plants on Lipid Peroxidation

Discussion

Phytochemical screening of the plant extracts revealed some difference between the plants. Flavanoids, coumarins, amino acids and proteins were observed in all the samples, while the other chemical showed a greater variation.

DPPH is a relatively stable free radical, it reacts with suitable reducing agents, the electrons become paired off and the solution loses colour stochiometrically depending upon the number of electrons taken up[16]. Greater levels of inhibition of free radicals in *P.granatum* may be related to certain characteristic phenolic compounds present in them. Perusal of literature reveals that flavanoids and tannins are potent free radical scavenging compounds. Flavanoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as a primary antioxidant or free radical scavenger. Hence this assay provided information on reactivity of the various extracts. Based on the result it is inferred that *P.granatum* extract is a potent agent for quenching the effect of free radicals. Earlier Anita and Jayashree[17] have also reported that many leafy vegetables and fruits of Indian regions are rich sources of antioxidants.

The aqueous extract of *P.granatum* showed broad spectrum of antimicrobial activity against all the three bacterial species studied. Based on the results obtained, it is concluded that *P.granatum* extract showed significant antioxidant and antimicrobial activity at very low concentration (150 µg/ml) compared to other extracts. In other plants antimicrobial activity was recorded only at higher (400 µg/ml) concentration. Low antimicrobial activity at higher concentration has also been recorded in *Mucuna pruriens*[15]. Several types of alkaloid, sterols and proteins known to exert antimicrobial activity[18, 19]. Therefore, the antimicrobial activity of the extracts might be due to the presence of flavanoids, alkaloids and antimicrobial substance. It is also reported[20] that leaf extracts contain less antimicrobial activity compared to other parts, due to the interference of pigments and phenolics with the antimicrobial activity of these extracts. It is obvious that the reduced antimicrobial activity recorded in extracts of other plants may be due to the presence of pigments like carotene and phenolics in leaf extracts. Since the selected medicinal plants contain varying levels of antioxidants and antimicrobial activity, their inclusion in day to day use is likely to be beneficial by reducing oxidative stress.

Since crude extracts are used in the present study, the components or active phytochemical constituents responsible for the various activities are not known. Therefore, thorough study is needed to isolate the active principles responsible for antioxidant and antimicrobial components especially in *Punicia granatum* and *Ruellia patula* for their industrial and pharmaceutical applications.

Conclusion

The present study showed that the extracts of *Punica granatum* possess greater DPPH scavenging activity compared to other plants tested. *Punica granatum* and *Ruellia patula* also showed significant *invitro* lipid peroxidation inhibition activities. The analysis of the aqueous extracts of the plants showed the presence of various classes of chemicals. Even though varied levels of anti-oxidant activity was present in all the 5 different plant species, significant antioxidant activity was recorded in *Punicia granatum*. Appreciable antimicrobial

activity was also observed in the extract of *P.granatum.* Hence it is concluded that inclusion of *P.granatum* in regular human use would be beneficial to reduce oxidation stress.

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