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IN VITRO INHIBITON OF LIPID PEROXIDATION AND ANTIMICROBIAL ACTIVITY OF *SAPINDUS TRIFOLIATUS* PERICARP

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Sapindus trifoliatus is a medium sized deciduous tree belonging to the family of *Sapindaceae*. Pericarp of the seeds of this is reported to possess various medicinal properties. The present investigation is aimed to carry out the, total antioxidant activity, *in vitro* inhibition of lipid peroxidation and antimicrobial activities of the ethanol extracts of *Sapindus trifoliatus* (EEST) pericarp. Antioxidant activity was determined by Ferric chloride reduced ability of plasma (FRAP) method. Inhibition of $FeSO_4$-ascorbate induced lipid peroxidation by EEST was determined and compared with standard antioxidants such as Quercetin, L-ascorbic acid. IC_{50} values for the EEST, L-ascorbic acid and Quercetin for lipid peroxidation were found to be 145µg/ml, 112µg/ml and 58µg/ml respectively. The antimicrobial activity of EEST was determined by agar well diffusion method with various Gram positive and Gram negative microorganisms. EEST showed broad spectrum of antimicrobial activity against all the tested microorganisms. The results of the present study indicate that EEST can be a potential source of antioxidant and antimicrobial agent.

Keywords: Antimicrobial activity; Antioxidants; *Sapindus trifoliatus*.

Introduction

Medicinal plants are good reservoirs of therapeutic compounds. There has been growing interest in the investigation of the natural products from plants for the discovery of new antioxidants and antimicrobial agents. In this quest, extracts of different plants are prepared and screened for their antioxidant and antimicrobial activities[1-3]. Because of the side effects and the resistance developed by pathogenic microorganisms against antibiotics, attention has been paid to isolate biologically active compounds from plant species used in herbal medicine[4]. Plant products represent a vast untapped resource of bioactive molecules, whose exploration is needed for antimicrobials and antioxidants. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are associated with synthetic antimicrobials[5].

Sapindus trifoliatus is a medium sized deciduous tree growing in south India that belongs to the family *Sapindaceae*. It is commonly known as soapnut tree. The pericarp is reported for various medicinal properties. Aqueous extracts of the pericarp is used for the treatment of hemicrania, hysteria or epilepsy, infectious diseases and migraine[6,7]. Phytochemical study revealed that the ethanol extract of *Sapindus trifoliatus* pericarp contained high amounts of saponins and Quercetin[8,9]. Phytochemicals such as alkaloids, phenolics, terpins and saponins are reported to posses antioxidant and antimicrobial activities[10]. Since pericarp is used in the treatment of many infectious diseases in traditional folk medicine and posses high amount of saponins and quercetin, the present investigation is aimed to carry out the antioxidant and antimicrobial activities of the ethanol extract of *Sapindus trifoliatus* pericarp.

Materials and Methods

All the chemicals used in the present study are of analytical grade and obtained from local suppliers.

Plant extract-The seeds of *Sapindus trifoliatus* were obtained from regional agricultural research station, Chinthapalli, Andhra Pradesh, India and authenticated by the Department of Botany, Andhra University. The seeds were thoroughly cleaned and the pericarp was removed, shade dried and powdered in a mechanical grinder. The sterilized powder of the pericarp is initially defatted with petroleum ether (60-80°C) and then extracted with 500ml of ethanol using a Soxhlet extractor for 72 hrs. The extract is filtered using Whatman (No1) filter paper and then

Table 1. Effect of EEST on some microorganisms.

Microorganism	Zone of inhibition (mm)		
	EEST (500µg/ml)	EEST (750 µg/ml)	Streptomycin (10 µg/ml)
E.coli	12±0.1	14±0.2	22±0.1
S. aureus	22±0.2	24±0.3	21±0.2
B. subtilis	25±0.2	26±0.2	18±0.1
K. pneumoniae	21±0.3	24±0.1	22±0.2
P.vulgaris	11±0.2	13±0.2	13±0.1
A. niger	12±0.1	15±0.1	18±0.2
C. albicans	10±0.2	12±0.2	22±0.2

All the values are an average of four determinations and expressed as mean ± S.D.

Table 2. Minimum inhibitory concentration of EEST.

Microorganism	Minimum inhibitory concentration (µg/ml)
E.coli	100±0.2
S. aureus	20±0.2
B. subtilis	50±0.2
K. pneumonia	30±0.2
P.vulgaris	150±0.2
A. niger	100±0.2
C. albicans	100±0.2

All the values are an average of four determinations and expressed as mean ± S.D.

concentrated in vacuum to dryness. Different concentrations of ethanolic extracts were prepared and used for the assay of antimicrobial activity, antioxidant activity and for *in vitro* lipid peroxidation.

Microbial strains employed- Microorganisms (*S. aureus, E. coli, B. subtilis, P. vulgaris, K. pneumoniae, A. niger* and *C. albicans*) were obtained from the department of Biotechnology, Andhra University and maintained on nutrient agar medium.

Anti microbiological assay- An aliquot of 0.1 ml of 1% $BaCl_2$ was added to 9.9 ml of 1% tetraoxosulfate to give a Mc Ferland turbidity standard suspension NO.1. This turbidity approximates bacterial density of about 3 x 10^8 organisms per ml. About 0.2 ml of the standardized suspension of the bacterial samples grown in nutrient broth were mixed evenly with molten agar and allowed to solidify. Antimicrobial activity of the extracts was tested using the agar well diffusion method[11]. 20 µl of extract was added to each well, allowed to diffuse into the surrounding medium for 1h and incubated at 37°C for 24 h. In order to compare the activity of the test material, Streptomycin (10 µg/ml) was used as positive control. After incubation, the zones of inhibition were measured. The minimum inhibition concentration (MIC) was determined as per the method described by *Raji et al.*[12].

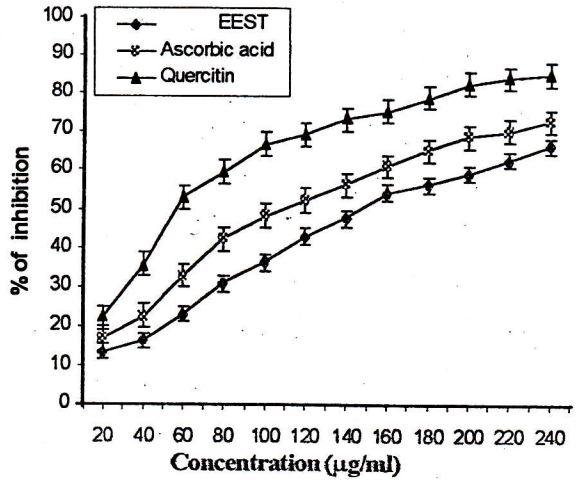

Fig.1. Effect of EEST and standard antioxidants on lipid peroxidation.

*Antioxidant assay-*Total antioxidant activity was determined by the modified FRAP (Ferric chloride reducing ability of plasma) method of Benzie and Strain[13]. In this assay, the FRAP reagent is prepared by adding 2, 4, 6-tripyridyl-s-triazine (TPTZ) and ferric chloride forming the Fe^{+3}-TPTZ complex. In presence of an antioxidant, the Fe^{+3}-TPTZ complex is reduced to Fe^{+2}-TPTZ complex which gives an intense blue color with maximum absorption at 595nm. The calibration curve was prepared using $FeSO_4$ with concentrations ranging from 0-1mM. The results are expressed as Ascorbic acid Equivalent Antioxidant Capacity (AEAC) units.

*In vitro inhibition of lipid peroxidation-*Lipid peroxidation induced by $FeSO_4$-ascorbate system in sheep liver homogenate by the method of Bishayee and Balasubramaniyam[14] was estimated as thiobarbituric acid reacting substances (TBARS) by the method of Ohkawa *et al.*[15]. The reaction mixture contained 0.1ml of sheep liver homogenate (25%) in Tris-HCl buffer (20mM, pH 7.0; KCl (30mM); $FeSO_4(NH_4)SO_4.7H_2O$ (0.06 mM) and various concentrations of *Sapindus trifoliatus* pericarp extract in a final volume of 0.5ml. The reaction mixture was incubated at 37°C for 1h. After the incubation, 0.4ml was removed and treated with 0.2ml sodium dodecyl sulphate (SDS) (8.1%), 1.5ml thiobarbituric acid (TBA) (0.8%) and 1.5ml of acetic acid (20%, pH 3.5). The total volume was made up to 4.0ml with distilled water and then kept in a water bath at 95°C for 1h. After cooling, 1.0ml of distilled water and 5.0ml of n-butanol and pyridine mixture (15:1) were added to the reaction mixture, shaken vigorously and centrifuged at 4000g for 10 min. The butanol pyridine layer was removed and its absorbance was measured at 532 nm.

Inhibition of lipid peroxidation was determined by comparing the optical density (OD) of the extract with that of the control. Quercetin and L-ascorbic acid were used as standard.

Statistical analysis-Each value is an average of four determinations. The statistical significance was evaluated by student's t-test and the values were expressed as mean ± SD. Level of significance was set at $p<0.05$.

Results and Discussion

The ethanol extracts of *Sapindus trifoliatus* pericarp showed significant antioxidant activity powder with a AEAC value of 0.056 mM. The effect of EEST and standard antioxidants namely Quercetin and ascorbic acid on the *in vitro* lipid peroxidation is shown in fig 1. The generation of lipid peroxidase by Fe^{2+} in sheep liver homogenate seems to be inhibited by EEST with IC_{50} value of 150µg/ml. A similar effect was observed with, L-ascorbic acid (IC_{50} = 112µg/ml) and Quercetin (IC_{50} = 58 µg/ml), indicating that the effect of EEST on the inhibition of lipid peroxidation is significant ($p<0.05$). The inhibition percentage of lipid peroxidation in the presence of extract was found to be 66.4%. The values for L-ascorbic acid and Quercetin were found to be 72.7% and 85.1% at 240µg/ml concentration.

The data presented in Table 1 indicate that the ethanol extracts of *Sapindus trifoliatus* pericarp inhibited the growth of the tested microorganisms to various degrees. The EEST at a concentration of 500µg/ ml and 750 µg/ ml exhibited significant ($p<0.05$) antimicrobial effect against all the tested microorganisms. The extract showed strong antimicrobial activity against *S. aureus* and *K. pneumoniae*. The antimicrobial activity was compared with the standard streptomycin at a concentration of10 µg/ml. The minimum inhibitory concentration of *S .trifoliatus* extract is given in Table 2. The results show that the MIC of *S .trifoliatus* is 20µg/ml against *S. aureus* and 30 µg/ml against *K .pneumoniae.*

The ethanol extracts of *Sapindus trifoliatus* pericarp showed comparable AEAC values with that of aqueous extracts of kokam[16]. Unsaturated lipids in liver tissue are highly susceptible to peroxidation when they are exposed to reactive oxygen species (ROS). In the present investigation we have incubated the liver tissue with $FeSO_4$ and examined the effect of extract on tissue homogenate by measuring the optical density (OD) at 532nm. The results of the investigations revealed that EEST had lipid peroxidation inhibitory activity.

Agar well diffusion method is extensively used to investigate the antimicrobial activity of natural substances and plant extracts. The antimicrobial activity of EEST was studied by the said method using various microorganisms. EEST showed a broad spectrum of antimicrobial activity against all the microbial strains studied. The zone of inhibition with EEST is greater than that of streptomycin (10 µg/ml) against *S. aureus, K .pneumoniae* and *B. subtilis*. Interestingly MIC for *S. aureus* and *K. pneumoniae* was found to be least with respect to other organisms considered under this study.

On the basis of the results obtained in the present study, it can be concluded that the ethanol extract of *Sapindus trifoliatus* has significant antioxidant and antimicrobial activities. Further studies are needed to isolate the active components, responsible for antioxidant and antimicrobial activities.

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