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Research Article

ANTIOXIDANT, CYTOPROTECTIVE AND ANTIINFLAMMATORY ACTIVITIES OF STEM BARK EXTRACT OF *SEMECARPUS ANACARDIUM*

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ABSTRACT

Reactive oxygen species (ROS) mediated oxidative damage has been implicated in the pathogenesis of number of disorders. Focus towards the natural sources of antioxidants has been increasing these days to fight against the deleterious effects of free radicals. *Semecarpus anacardium,* medicinal plant of *Anacardiaceae* family used as remedy in the alleviation of disorders. The present study was focused to evaluate the antioxidant, cytoprotective and anti-inflammatory potentials of methanolic extract of *S.anacardium stem bark* by most accepted methods. The methanolic extract exhibited significant DPPH, superoxide and hydroxyl radical scavenging activities. In addition, the methanolic extract also exhibited significant nitric oxide scavenging ability. The extract showed significant protection against Fenton reaction induced lipid peroxidation in sheep liver tissue homogenate model, heat induced hemolysis in human RBC membrane model, inhibition of heat induced protein denaturation and proteinase inhibitory activity.

Keywords: *Semecarpus anacardium*, radical scavenging, natural antioxidant, antioxidant activity, cytoprotective activity, anti inflammatory activity.

INTRODUCTION

Reactive oxygen species (ROS) play a key role in the prevalence of diseases at both cellular and molecular levels[1-2]. It is increasingly realized that many of today's diseases are due to the ROS mediated damage of biological macromolecules such as proteins, lipids and DNA in cells. These changes can contribute to atherosclerosis, cardiovascular and inflammatory diseases and cancer[3]. Inflammation can be initiated either by injury or infection. Activation of phagocytes leads to the production of radical and non radical species of both oxygen and nitrogen, which affects surrounding tissue either directly or indirectly. This initiates lipid per oxidation resulting in membrane destruction of cells and cell organelles. Lysosomal enzymes also released during inflammation produces a variety of disorders which leads to the tissue injury by damaging the macromolecules and lipid per oxidation of membranes. The extra cellular activity of these enzymes is said to be related to acute or chronic inflammation. In addition the reactive oxygen and nitrogen species are also known to activate several proteases causing increased destruction of tissues in various inflammatory disorders [4]. The production of auto-antigens in certain inflammatory diseases may be due to denaturation of proteins and the mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding[5].

Stabilization of lysosomal membrane is important in limiting the inflammatory response of reactive species[6]. Erythrocyte membrane is structurally analogous to the lysosomal membrane. Therefore, the use of erythrocyte membrane is good model to study the protective effect of medicinal plant extracts[7]. Previously, stabilization of hypo tonicity induced human red blood cell membrane (HRBC) was used as an *in vitro* model to study the cytoprotective activity of medicinal plant extracts [8].

The uses of medicinal plants as traditional medicine is wide spread and represent a large source of natural anti-oxidants that might serve as leads for the development of the novel drugs [9].Recently, much attention has been directed towards the development of ethno-medicines with strong antioxidant properties[10].

Semecarpus anacardium (*Anacardiaceae*) commonly known 'Ballataka' or 'Bhilwa' is a medium to large size tree. It is well-known for medicinal value in ayurvedic and siddha system of medicine[11]. Different parts of this plant have been traditionally used to treat rheumatism, asthma, neuralgia, helminthic infection, psoriasis and cancer[12]. Earlier, the aqueous stem bark extract was reported to have antimicrobial, CNS stimulant, hypoglycemic, anti-atherogenic and anti-carcinogenic activities[13]. The ethyl acetate extract of stem bark showed inhibitory activity on pro-inflammatory enzymes such as cyclooxygenase and acetyl cholinesterase[14-15]. However, antioxidant and anti-inflammatory activities of methanolic extracts of stem bark was not explored. The present study was focused to evaluate the antioxidant and anti-inflammatory potentials of methanolic extract of *S.anacardium* stem bark by widely accepted methods.

MATERIALS AND METHODS

Thiobarbituric acid (TBA), 1,2-dipheny1-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-striazine (TPTZ), nitroblue tetrazolium (NBT), were purchased from Sigma, USA. Quercetin, ascorbic acid, butylated hydroxyl toluene(BHT), sodium acetate, ferric chloride, tris-Hcl, ferrous sulphate, sodium dodecyl sulphate (SDS), acetic acid, butanol, pyridine, deoxyribose, EDTA, H_2O_2, tricholoro acetic acid (TCA), potassium ferro cyanate ($K_3[Fe(CN)_6]$, sodium carbonate, hydroxylamine hydrochloride, sodium azide (NaN_3), albumin, diclofenac sodium, aspirin were purchased from Merck, Germany. The other chemicals and solvents used in the present study were of analytical grade obtained from local supplier in pure quality.

Collection of plant material

The stem bark of *S. anacardium* was collected from the Eastern Ghats of Vizianagaram region and authenticated by the faculty in the department of Botany, Andhra University, Visakhapatnam.

Preparation of plant extract

The stem bark was thoroughly cleaned, shade dried and powdered in a mechanical grinder. The powder was extracted with soxhlet extractor using n- hexane, ethyl acetate, methanol and water for 48 hrs. The extracts were concentrated to dryness in desiccator. The solvent extracts were used to determine the DPPH radical scavenging ability and total antioxidant activity. Further, different concentrations of methanolic extract (25,50,100,250,500,750 and 1000µg) were prepared in aforesaid solvents and were used to assay antioxidant, cytoprotective and anti-inflammatory activities.

Antioxidant assays

Determination of total antioxidant activity

The total antioxidant status of stem bark extract of *S.anacardium* was assayed by using FRAP method as described Wong *et al*. with some modifications[16]. Briefly, 0.2ml of different concentrations (25, 50,100,250,500,750 and 1000µg/ml) of methanolic extracts were added to 3.0 ml of FRAP reagent (mixture of 300 mM sodium acetate buffer (pH 3.6), 10 mM, TPTZ solution and 20 mM $FeCl_3$ in a ratio of 10:1:1. The reaction mixture was incubated in a water bath at 37°C for 30 min. The increase in the absorbance was measured using

spectrophotometer at 593 nm. The total antioxidant capacity was assessed based on the ability to reduce ferric ions by the extracts. The percent of total antioxidant activity was calculated using a formula,

Percent of antioxidant activity = [(A_{593} of sample – A_{593} of control)/ A_{593} of sample] x 100

DPPH radical scavenging activity

DPPH scavenging activity was measured by the method of Cuendet *et al*[17]. To 3.0 ml of methanolic solution of DPPH (0.1mM), 1.0 ml of extract was added. In control, the extract was replaced by methanol. The reaction mixture was incubated for 30 min at 37^{0}C and absorbance was measured at 517nm using UV-visible spectrophotometer. The percentage of inhibition was calculated from the following equation: A_0-Ax100/A_0, where A_0 and A are the absorbance of control and test sample, respectively. BHT and Quercetin were used as standards.

Determination of Superoxide radical scavenging activity

The superoxide radical scavenging ability of stem bark extract of *S.anacardium* was determined by the method of Beauchamp and Fridovich[18]. To 0.5ml of extract, 1.0 ml of 0.12 M sodium carbonate, 0.4ml of 25µM NBT and 0.2ml of 0.1mM EDTA were added. The reaction was initiated by adding 0.4ml of 1.0mM hydroxylamine hydrochloride and incubated for 20 min. The absorbance was measured at 560nm using spectrophotometer. The super oxide anion scavenging activity is calculated as percent inhibition of absorbance compared to the control.

Determination of hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was carried out by measuring the competition between deoxyribose and the extract for hydroxyl radical's generated by fenton reaction, a method originally described by Gulhan *et al*.,19. 0.1ml of plant extract was added to the reaction mixture containing 0.1ml of 3.0 mM deoxyribose, 0.5 ml of 0.l m M $FeCl_3$, 0.5 ml of 1mM H_2O_2 and 0.8 ml of 20 mM phosphate buffer, pH 7.4 in a final volume of 3.0ml and incubated at 37°C for 1hr. The thiobarbituric acid reactive substances (TBARS) formed were measured by treating with 1.0 ml of TBA (1.0%) and 1.0 ml of TCA (2.8%) at 100°C for 20min. After the mixtures were cooled, absorbance was measured at 532 nm against control, which is devoid of plant extract. Percentage of inhibition was calculated as

(I) = [(Absorbance of control- Absorbance of test / Absorbance of control)] x 100.

Determination of Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity of the extract is estimated by the method of Zhang[20]. 1.0ml of 0.1mM H2O2 and 1.0ml of various concentrations of plant extract or BHT or Quercetin are mixed, followed by 2 drops of 3% ammonium molybdate, 10ml of 2M H2SO4 and 0.7 ml of 1.8M KI. The mixed solution is titrated with 5.09mM Na2S2O3 until yellow color is disappeared. For control, all reagents are added except plant extract. Percentage of scavenging of hydrogen peroxide is calculated as percent inhibition.

Cytoprotective assays

Inhibition of lipid peroxide formation

Lipid peroxidation induced by FeSO4- ascorbate system in sheep liver homogenate is estimated as thiobarbituric acid reacting substances (TBARS) by the method of Ohkawa, Ohishi & Yagi[21]. The reaction mixture contained 0.1ml of sheep liver homogenate (25%) in Tris-HCl buffer (20mM, pH 7.0; KCl (30mM); FeSO4 (NH4) SO4.7H2O (0.06 mM) and various concentrations of plant extracts and positive control in a final volume of 0.5ml and incubated at 37oC for 1h. After the incubation, 0.4ml is removed and treated with 0.2ml sodium dodecyl sulphate (8.1%), 1.5ml thiobarbituric acid (TBA) (0.8%) and 1.5ml of trichloroacetic acid (20%). The total volume is made up to 4.0ml with distilled water and then kept in a water bath at 95oC for 1h. After cooling, 1.0ml of distilled water and 5.0ml of n-butanol and pyridine mixture (15:1) are added to the reaction mixture, shaken vigorously and centrifuged at 4000g for 10 min. The butanol pyridine layer is removed and its absorbance is measured at 532 nm. Control is also run in the same manner but plant extract is replaced with methanol. Inhibition of lipid peroxidation is determined by comparing the optical density (OD) of the test sample with that of the control.

Percent of inhibition of lipid peroxidation (I) = [(A_{532} of control- A_{532} of sample / A_{532} of control)] x 100.

HRB membrane stabilization test

HRB membrane stabilization test was performed by the following described method[22].

Fresh whole human blood (10ml) was collected and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10min and were washed three times with equal volume of normal saline. The volume of blood was measured and re constituted as 10% v/v suspension with normal saline. The reaction mixture 2ml consists of 1 ml of test sample solution and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Aspirin was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56^{0}C for 30min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples. Percent membrane stabilization activity was calculated by the formula

Percentage Inhibition = (A of Control – A of Sample)/A of Control x100.

Anti-inflammatory assays

Nitric oxide scavenging activity

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH was measured by Griess reaction[23]. The reaction mixture (3ml) containing sodium nitropruside (10mm) in phosphate buffer saline and the test extract (10, 25, 50 and 100µg/ml) was incubated at 25^{o}C for 150min , after incubation 1.5ml of the reaction mixture was removed and 1.5ml of the Griess reagent (1% sulphanilamide, 2% orthophosphoric acid and 0.1% Napthylethyline diamine hydrochloride) was added. The absorbance of the chromophore formed was read at 546 nm. Percent inhibition of nitric oxide scavenging was calculated using the formula.

Percentage Inhibition = (A of Control – A of Sample)/A of Control× 100. A- absorbance.

Inhibition of albumin denaturation

Method of Mizushima *et al* was followed with minor modifications[24]. The reaction mixture was consisting of test extracts at different concentrations and 1% aqueous solution of bovine albumin fraction. pH of the reaction mixture was adjusted using small amount of 1N HCl. Diclophenac sodium was taken as standard drug. The samples were incubated at 37^{o}C for 20 min and then heated at 57^{o}C for 30 min. After cooling the samples, the turbidity was measured spectrophotometrically at 660 nm. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows:

Percentage Inhibition = (A of Control – A of Sample)/A of Control x100

Proteinase inhibitory activity

Proteinase inhibitory activity was performed according to the modified method of Oyedepo *et al*[25]. The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1 ml 20 mM Tris HCl buffer (pH 7.4) and 1 ml test sample of different concentrations. The mixture was incubated at 37^{o}C for 5 min. The 1 ml of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min. Then 2 ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged at 3000 rpm for 10 min. The absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage

of proteinase inhibitory activity was calculated using the following formula.

Percentage Inhibition = (A of control – A of sample)/A of control x100.

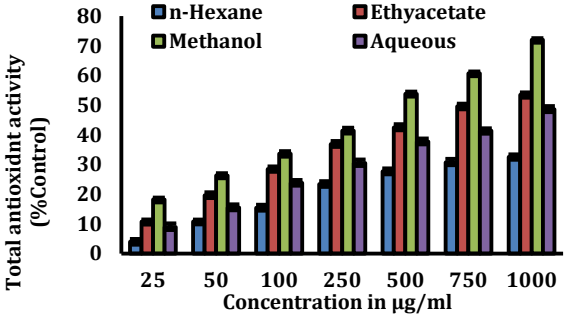
Statistical analysis

The experiments were replicated thrice for each parameter and the standard deviation was calculated.

RESULTS AND DISCUSSION

Reactive oxygen species (ROS) are produced continuously in most tissues and are inextricably linked to malignant diseases, diabetes, atherosclerosis, chronic inflammation and ischemia-reperfusion injury[26]. Superoxide and hydroxyl radicals, hydrogen peroxide, singlet oxygen are known to be cytotoxic and have been implicated in the etiology of various diseases[27]. Epidemiological and *in vitro* studies have revealed that medicinal plant extracts protects biological systems from oxidative stress[28]. *S.anacardium* is a well known medicinal plant and traditionally used for the treatment of stress mediated disorders. In the present study, total antioxidant and antiradical activities were used to determine antioxidant capacity of stem bark extract *S.anacardium* due to its differential mechanisms of antioxidant action.

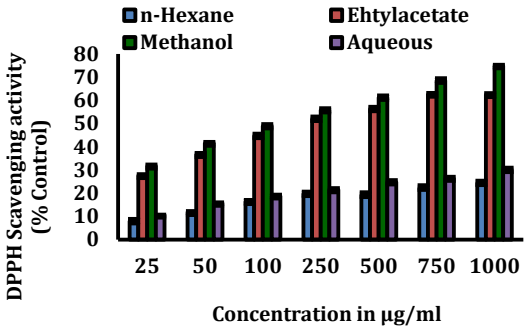
The FRAP assay is a simple, convenient and reproducible method widely employed to determined the total antioxidant activity of biological samples[29]. As shown in the figure-1a, methanol, ethyl acetate, aqueous and n-hexane extracts of *S.anacardium* stem bark exhibited 72.00, 53.48, 48.62 and 32.58%, respectively, at 1mg/ml. Ascorbic acid represented the standard showed 78.5% total antioxidant activity at 1000 µg/ml. The results from this antioxidant assay suggested that the methanolic extract showed higher FRAP reducing activity than other solvent extracts. The significant FRAP of methanolic extract may be due to extraction of more hydrogen donation compounds by methanol than other solvents used in the present study. FRAP is reduced by the compounds that can donate hydrogen atoms to free radicals and convert them into stable non-reactive molecules[30].



Total antioxidant activtity of n-Hexane, ethylacetate, methanol and aqueous extracts of stem bark of *S.anacardium*. Each value is expressed as mean ± standard deviation (n=3). Concentration (µg/ml) taken on X-axis and % inhibition taken on Y-axis.

Fig 1a: Total Antioxidant Activity

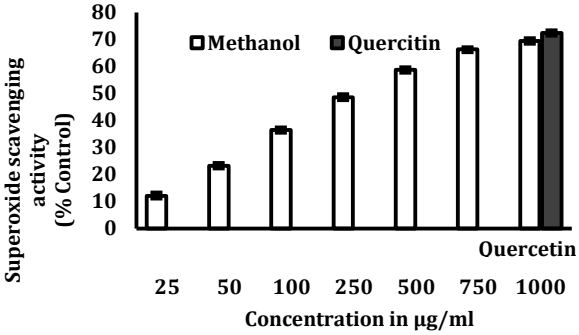
Oxygen derived radicals represent the most important class of radical species generated in living systems[31]. The harmful effect of free radicals causing potential biological damage is oxidative stress[32]. DPPH is stable and non-physiological radical and most widely used method for screening antioxidant activity of plant extracts[33]. DPPH is reduced to diphenylpicryl hydrazine with plant extracts in a concentration-dependent manner. The radical scavenging ability of different solvent extracts on DPPH radical is in the following order: methanol > ethyl acetate > aqueous >hexane with 74.85, 62.45, 30.12 and 24.52% respectively, at 1000 µg/ml concentration(Fig. 1b). Among the four extracts, methanol extract had shown the highest DPPH scavenging activity similar to natural antioxidant Quercetin(78.35%). As methanolic extract exhibited significant as it is exhibited significant antioxidant activity and DPPH radical scavenging activity, further studies were carried out with methanolic extract.



DPPH Scavenging of n-Hexane, ethylacetate, methanol and aqueous extracts of stem bark of *S.anacardium* . Each value is expressed as mean ± standard deviation (n=3). Concentration (µg/ml) taken on X-axis and % inhibition taken on Y-axis.

Fig 1b: DPPH Scavenging Activity

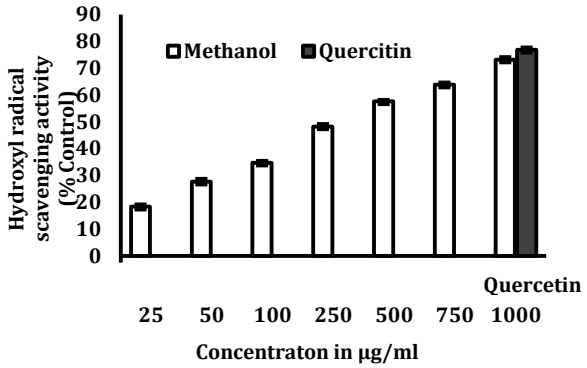
Super oxide is biologically important radical as it can form singlet oxygen and hydroxyl radical which can contributes to the pathogenesis of many diseases. Over production of super oxide anion radical contributes to redox imbalance and associated with harmful physiological consequences[34]. Super oxide anions can be generated artificially from the hydroxylamine/ EDTA system. The scavenging ability of extracts can be assayed by NBT reduction method. The superoxide scavenging ability of the ethanolic extract of stem bark of *S.anacrdium* was found to be 69.45% compared to control, whereas known antioxidant Quercetin exhibited 72.45% at 1000 µg/ml. (Fig. 2a). The IC_{50} values of stem bark extract and Quercetin were 240 and 215 µg/ml, respectively(Table- 1).These results indicate that the crude methanolic extract of stem bark exhibited nearly equal to the pure natural compound Quercetin.



Superoxide radical scavenging activity of methanolic stem bark extract of *S.anacardium* compared to that of standard Quercetin(1mg/ml). Each value is expressed as mean ± standard deviation (n=3). Concentration (µg/ml) taken on X-axis and % inhibition taken on Y-axis.

Fig 2a: Superoxide Scavenging Activity

Hydroxyl radical is the most deleterious and reactive radical among the ROS with shortest half-life compared to other free radicals.The oxygen derived hydroxyl radicals in presence of transition metal ion (Fe2+) causes the degradation of deoxyribose into malondialdehyde which produces a pink chromogen with thiobarbituric acid[35]. The methanolic extract of stem bark of *S.anacardium* exhibited hydroxyl radical scavenging activity in a dose dependent manner and significant inhibition was observed at 1000 µg/ml concentration with (73.20%) IC_{50} value of 235 µg/ml. The crude methanolic extract exhibited comparable percent of inhibition with and natural antioxidant Quercetin with 76.85% with IC_{50} value 210 µg/ml (Fig. 2b).



Hydroxyl radical scavenging activity of methanolic stem bark extract of *S.anacardium* compared to that of standard Quercetin(1mg/ml). Each value is expressed as mean ± standard deviation (n=3). Concentration (µg/ml) taken on X-axis and % inhibition taken on Y-axis.

Fig 2b : Hydroxyl Radical Scavenging Activity

Hydrogen peroxide is a nonradical reactive oxygen species with weak oxidizing activity.

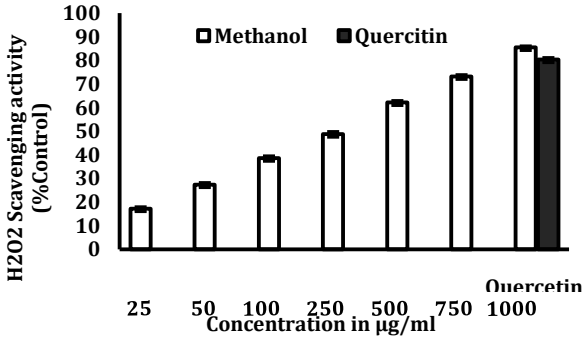
It diffuse through cell membranes rapidly and interacts with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radicals and other free radicals[36]. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. The hydrogen peroxide scavenging ability of methanolic extract of stem bark of *S.anacardium* is shown in the figure-2c, and the results show that methanol extract exhibited significant hydrogen peroxide scavenging activity in a concentration dependent manner with highest activity at 1000 µg/ml (85.45%) compared to Quercetin (80.32%).These results indicate that the methanolic extract exhibited high percent of inhibition comapared to Quercetin, a known antioxidant. The IC_{50} values of Plant extract and Quercetin were found to be 215 and 260 µg/ml, respectively(Table-1).

Table-1: IC_{50} Values Of Methanolic Extract Compared Against Respective Standards

MODEL	IC_{50} value of Methanolic extract (µg/ml)	IC_{50} value of Standard(µg/ml)
Antioxidant assays		
Superoxide scavenging activity	240	215
Hydroxylradical scavenging activity	235	210
Hydrogen peroxide scavenging activity	215	260
Cytoprotective assays		
Inhibition of Lipid peroxidation activity	325	345
HRBmembrane stability assay	190	215
Anti-inflammatory assays		
Nitricoxide scavenging activity	255	210
Inhibition of Protein Denaturation activity	250	220
Proteinase inhibitory activity	210	205

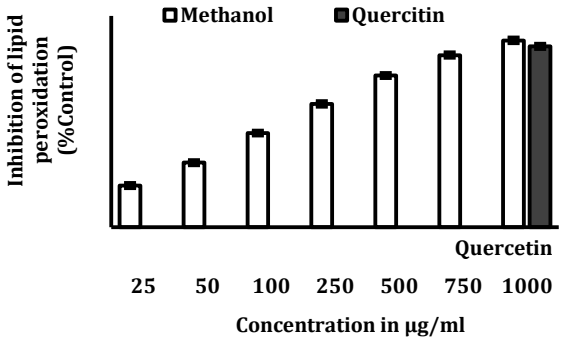
The cytoprotective activity of methanolic extract was determined in terms of inhibition of sheep liver membrane lipid peroxidation and human RBC membrane stabilization assays.

Lipid peroxidation is a well-established mechanism of cellular injury in both plants and animals and is used as an indicator of oxidative stress in cells and tissues[37]. The measurement of TBARS substances such as malondialdehyde (MDA) has been employed to monitor membrane damage by various reactive oxygen species[38].The results showed that the methanol extract of *S.anacardium* inhibited FeSO4 induced lipid peroxidation of sheep liver tissue homogenate in a dose dependent manner(Fig. 3a). Maximum inhibition was observed at 1000µg/ml with 70.65%(IC_{50} 325) which was higher than the standard antioxidant Quercetin activity (68.45%) with IC_{50} value 345µg/ml. Thus the decrease in the MDA level in sheep liver homogenate with the increase in concentration indicates the cytoprotective role of the plant extract.



Hydrogen peroxide scavenging activity of methanolic stem bark extract of *S.anacardium* compared to that of standard Quercetin(1mg/ml). Each value is expressed as mean ± standard deviation (n=3). Concentration (µg/ml) taken on X-axis and % inhibition taken on Y-axis.

Fig 2c: Hydrogen Peroxide Scavenging Activity

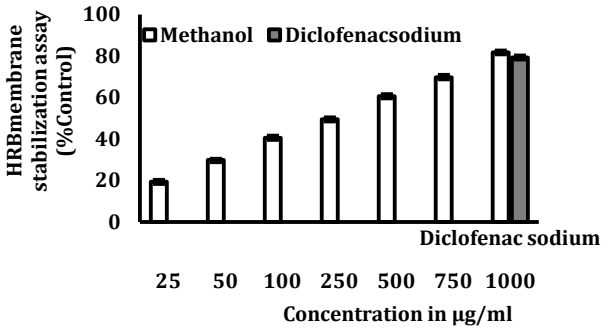


Inhibition of lipid peroxide formation ability of methanolic stem bark extract of *S.anacardium* compared to that of standard Quercetin(1mg/ml). Each value is expressed as mean ± standard deviation (n=3). Concentration (µg/ml) taken on X-axis and % inhibition taken on Y-axis.

Fig 3a : Lipid Peroxidation Assay

The HRBC membrane stabilization assay has been used to study the cytoprotection of erythrocyte membrane[39].

Stabilization of the RBCs membrane was studied to further establish the mechanism of cytoprotective activity of *S.anacardium* extract. The extract was effective in inhibiting the heat induced hemolysis at different concentrations. They provide evidence for membrane stabilization as an additional mechanism of their cytoprotective effect. The plant extract inhibited the heat induced hemolysis of RBCs to varying degree at 1000 µg/ml in (Fig. 3b). The methanolic extract of *S.anacardium* exhibited maximum activity 81.85% at 1000µg/ml with IC50 value 190 µg/ml. Non steroidal anti-inflammatory drug, diclofenac sodium exhibited 79.35% of cytoprotective activity at 1000µg/ml with IC50 value 215 µg/ml(Table-1). Although the precise mechanism of this membrane stabilization is yet to be elucidated, it is possible that the methanolic extract of stembark of *S.anacardium* produced this effect surface area/volume ratio of the cells, which could be brought about by an expansion of membrane or the shrinkage of the cells and an interaction with membrane proteins[40].



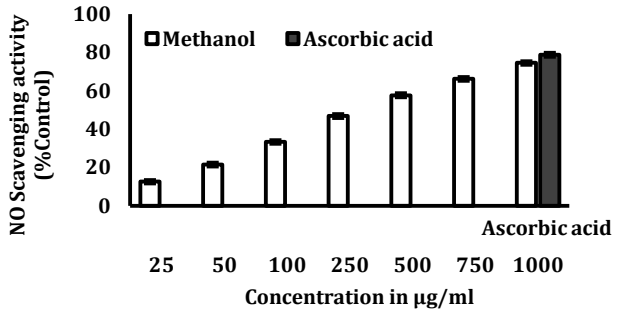
HRB membrane stabilization ability of methanolic stem bark extract of *S.anacardium* compared to that of standard drug Diclofenacsodium(1mg/ml). Each value is expressed as mean ± standard deviation (n=3). Concentration (µg/ml) taken on X-axis and % inhibition taken on Y-axis.

Fig 3b : Hrb Membrane Stabilization Assay

Inflammation is a bodily response to injury, infection or destruction characterized by heat, redness, pain, swelling and disturbed physiological functions[41].The commonly used drugs for management of inflammatory conditions are non-steroidal anti-inflammatory drugs, which have several adverse effects especially gastric irritation leading to formation of gastric ulcers[42].Therefore, search for natural antioxidants with anti inflammatory activity has been greatly increased in the recent years[43].

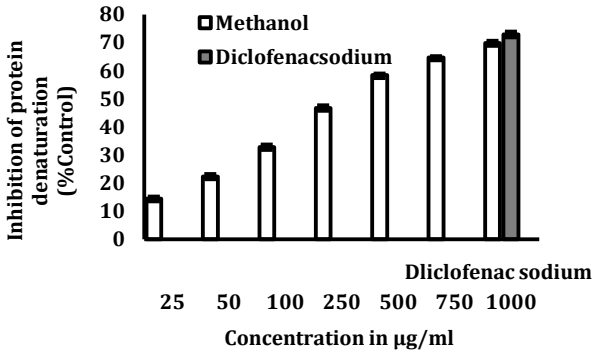
Nitric oxide (NO) is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity[44]. It is a diffusible signaling molecule considered as proinflammatory mediator that plays a key role in the pathogenesis of inflammatory disorders[45]. Generally scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions[46]. Methanol extract of *S.anacardium* exhibited significant nitric oxide scavenging activity in dose dependent manner with percent of inhibition 74.58 at 1000µg/ml concentration compared with Ascorbic acid (78.75%) with IC_{50} values 255 and 210 µg/ml, respectively (Fig. 4a).

Denaturation of proteins is a well-documented cause of inflammation. Several anti-inflammatory drugs have shown dose dependent ability to inhibition of protein denaturation[47]. As a part of the investigation on the mechanism of the anti-inflammatory activity, ability of extracts to inhibit protein denaturation was studied. From the results, it was found that the ethanol stem bark extract of *S.anacardium* was effective in inhibiting heat induced albumin denaturation at different concentrations(Fig. 4b). The methanolic extract of *S.anacardium* showed maximum inhibition 69.89% at 1000µg/ml with IC_{50} value 250 µg/ml. Diclofenac sodium is anti-inflammatory drug represented the positive control had exhibited the inhibitory activity 72.92% at 1000µg/ml with IC_{50} value 220 µg/ml(Table-1).



Nitric oxide scavenging activity of methanolic stem bark extract of *S.anacardium* compared to that of standard Ascorbic acid(1mg/ml). Each value is expressed as mean ± standard deviation (n=3). Concentration (µg/ml) taken on X-axis and % inhibition taken on Y-axis.

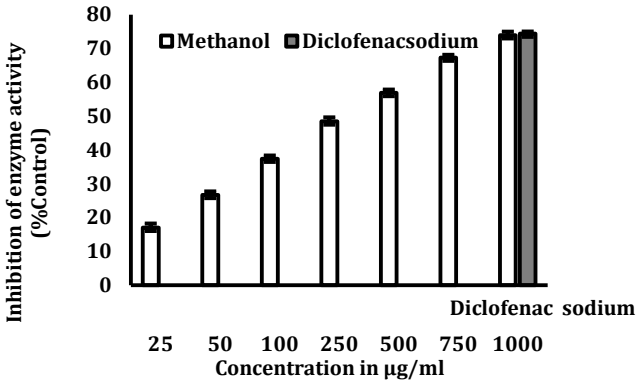
Fig 4a: Nitric Oxide Scavenging Activity



Protein denaturation inhibitory activity of methanolic stem bark extract of *S.anacardium* compared to that of standard Diclofenac sodium(1mg/ml). Each value is expressed as mean ± standard deviation (n=3).Concentration (µg/ml) taken on X-axis and % inhibition taken on Y-axis.

Fig 4b : Protein Denaturation Inhibition Assay

Proteinases have been implicated in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors[48]. The results indicates that the methanloic extract of *S.anacardium* bark inhibited the proteinase trypsin in dose dependent manner displaying the most potent inhibitory activity with 73.95% at 1000 µg/ml (IC50 210 µg/ml) while diclofenac sodium represented the positive control had exhibited the 74.42% with IC50 205 µg/ml(Fig. 4c).



Trypsin inhibitory activity of methanolic stem bark extract of *S.anacardium* compared to that of standard Diclofenac sodium(1mg/ml). Each value is expressed as mean ± standard deviation (n=3). Concentration (µg/ml) taken on X-axis and % inhibition taken on Y-axis.

Fig 4c : Proteinase Inhbitiory Activity

CONCLUSION

From these results, it is concluded that the methanolic extract of *S.anacardium* stem bark, exhibits high antioxidant, free radical scavenging activities. It also exhibited significant cytoprotective and anti-inflammatory activities against standards. The results also indicate that plant extract is a significant source of natural antioxidant, which might be helpful in preventing the progression of various oxidative stress mediated disorders. Further investigation on isolation of antioxidant compounds from methanolic plant extract is under progression.

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