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PROTECTION AGAINST OXIDATIVE STRESS BY *SEMECARPUS ANACARDIUM*

Wani A. S., Inamdar T.N., Kashid R.M., Shahane S.A., Moharekar S.T., Patil M.T. and Patil B.A.
Department of Biotechnology, New Arts, Commerce and Science College,
Ahmednagar (M.S.) India
(E-mail- ashishwani19@gmail.com)

ABSTRACT
In this study *Semecarpus anacardium* nut was analyzed for phytochemicals, antioxidant activity and lipid peroxidation inhibiting activity. It revealed that extracts contain various phytochemicals like alkaloids, glycosides, saponins, tannins, flavonoids and steroids. Total flavonoid content was higher in aqueous extract of *S. anacardium* (1.042 mgQE/gm). Also phenol content was higher in ethanolic extract of *S.anacardium* (0.507 mgGAE/gm). Antioxidant activity of chloroformic extract (IC_{50}-21.27 µg/ml) was almost similar to the standard ascorbic acid (IC_{50}-28.19 µg/ml). Inhibition of lipid peroxidation in brain tissue homogenate analysed by TBARS assay found to be higher by ethanolic extract of *S. anacardium* (30.54%). This result explain the ethanobotanical uses of the *S. anacardium* to alleviate diseases caused due to the oxidative stress.

KEYWORDS: DPPH, Lipid peroxidation, Oxidative stress, phytochemical analysis.

INTRODUCTION

Oxidative stress, in which the extracellular and intracellular production of reactive oxygen species (ROS) overwhelms physiological antioxidant defenses, it initiates damage to lipids (lipoperoxidation), proteins and DNA (Fallarini *et al*., 2009). Reactive Oxygen Species (ROS) denote a collection of oxygen radicals (O_2^-, OH*), and some derivatives of oxygen like H_2O_2 and singlet oxygen. ROS are generated during the normal metabolism of eukaryotic cells, which involve mitochondrial electron transport, microsomal P_{450} and other systems (Maleyki *et al.*, 2008; Rani *et al*., 2004). Antioxidants are those compounds that prevent the damage done to cells by free radicals that are released during the normal metabolic process or under stressful conditions. Antioxidants protect unsaturated fats in the body from oxidation by peroxides and other free radicals (Habila *et al.*, 2010). The antioxidant enzymes and free radical scavengers provide a defensive mechanism against the deleterious actions of ROS. Some of the antioxidant enzymes that are found to provide a protection against the ROS are superoxide dismutase, catalase, peroxidase, glutathione peroxidase (GPx), glucose-6-phosphate dehydrogenase and ascorbate oxidase. The non-enzymatic antioxidants which act as scavengers are glutathione, vitamin A, vitamin E, and vitamin C. The antioxidants may be of either the natural ones or the synthetic ones. Commonly used synthetic antioxidants are: butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), propyl gallate and tertiary butyl hydroquinone. While the naturally occurring antioxidants like vitamins are a balanced mixture of redox with reduced and oxidized form, the synthetic antioxidants are unbalanced in this respect and they themselves produces harmful free radicals in some cases, emphasizing the importance of the naturally occurring antioxidants over the synthetic ones (Rani *et al*., 2004). Despite emphasis being put in research of synthetic drugs, a certain interest in medicinal plants has been reborn, in part due to the fact that a lot of synthetic drugs are potentially toxic and are not free of side effects on the host (Dangoggo *et al.*, 2012).

The nervous system is extremely vulnerable to a wide range of harmful stimuli, such as oxidative stress, excitotoxicity and is particularly susceptible to irreversible damages because it has a reduced capacity for regeneration. Substantial pieces of evidence have mounted over the years showing the biochemical and cellular events associated with neuronal death. Common players in necrotic cell death, irrespective of the specific stimulus, are oxidative stress and Ca^{2+} overload. Recently, the scientific interest in this field has increasingly focused on natural products. Phytochemicals, including vitamin C, vitamin E, ß-carotene and phenolic compounds, are endowed with cytoprotective effects, and the consumption of food containing these compounds has been proposed for the prevention of pathological (Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, stroke, retinal degeneration) or physiological (aging) neuronal death. Among phytochemicals, phenolic compounds are an important class of quasi-vitamins of potential use at the nutritional/pharmaceutical interface (Fallarini *et al*., 2009).

S. anacardium Linn (*Family: Anacardiaceae*) is distributed in sub-Himalayan region, tropical and central parts of India. The nut is commonly known as 'marking nut' and in the vernacular as 'Ballataka' or 'Bhilwa'. It has high

priority and applicability in indigenous system of medicine. Nut shells contain the biflavonoids: biflavones A, C, A1, A2, tetrahydrorobustaflavone, B(tetrahydromentoflavone), jeediflavone, semecarpuflavone and gulluflavone. Oil from nuts , bhilavinol, contains a mixture of phenolic compounds mainly of 1,2-dihydroxy-3(pentadecadienyl-8,11) benzene and 1,2-dihydroxy-3 (pentadecadienyl-8', 11')- benzene (Majumdar *et al.*, 2008; Sahoo *et al.*, 2008).The aim of our study was to evaluate potential of *S. anacardium* nut against oxidative stress.

MATERIALS AND METHODS

1. Collection of plant material:

The nuts of *S. anacardium* were collected from Western Ghat Region of Maharashtra. The nuts were identified and authenticated by Department of Botany, New Arts, Commerce and Science College, Ahmednagar. The nuts of *S. anacardium* were shade dried for 15 days in laboratory. The powder was made from the dried nuts in the grinder. The powdered sample was used for further analysis.

2. Preparation of Plant Extract:

Extraction of sample was carried out by using three solvents- chloroform, ethanol and water. The powder was dissolved in solvent at the ratio 1:10 in the flask and kept at room temperature for 72 hrs with occasional gentle shaking. After 72 hours, the residue was removed by Whatman filter paper and the filtrate was evaporated to dryness (Dangoggo *et al.*, 2012; Parthasarathy *et al.*, 2009).

3. Phytochemical analysis:

i) Qualitative analysis - Phytochemical screening of the extracts was carried out according to the methods described by Trease and Evans for the detection of active components like saponins, tannins, alkaloids, phlobatanins, glycosides (Trease and Evans, 1989).

ii) Determination of Total Phenolics:

Determination of total phenolics was done using Folin-Ciocalteu method(Habila *et al.*, 2010). To each 1 ml plant sample solution (0.1mg/ml) and the standard (gallic acid) with different concentration-20,40,60,80,100 µg/ml was added 5ml of Folin-Ciocalteu and 4 ml sodium carbonate (7% w/v) and shaken. The solution was allowed to stand for 30 min. in the dark at room temperature, after which absorbance was measured at 765 nm using a spectrophotometer. The amount of total phenolics was expressed as gallic acid equivalent (GAE) in milligram per gram dry plant extract using the expression;

$$\text{Total phenolic content} = c \times V/m$$

Where c is Gallic Acid Equivalence (mg/ml) or concentration of gallic acid established from the calibration curve; V is the volume of extract in ml and m is the weight of pure plant extract (g).

iii) Determination of Total Flavonoid :

The method described by Mohamed et al was used as a guideline to carry out this determination. Quercetin was prepared in various concentrations (20µg-100 µg) in methanol. 0.5 ml of each extract (0.1 mg/ml) were taken in the test tube and 1.5ml methanol, 0.1ml 10% aluminium chloride, 0.1ml of 1M potassium acetate and 2.8ml of distilled water were added separately to each tube. All tubes were incubated at room temperature for 30 minutes. O.D. was measured at 415nm by using spectrophotometer (Parthasarathy *et al.*, 2009). Results were expressed as milligrams of Quercetin equivalent per gram of dry weight (mg QE/g) of extracts. Total content of flavonoid compounds in the plant extract was calculated using this formula:

$$\text{Total flavonoid content} = QE * V/m$$

Where QE, is the quercetin equivalence (mg/mL) or concentration of quercetin solution established from the calibration curve; V, is the volume of extract (mL) and m, is the weight of the pure plant extract (g).

4. Antioxidant activity by DPPH method:

Antioxidant activity was determined by Diphenylpicrylhydrazyl (DPPH) assay (Molyneux, 2004). Different concentrations of the test sample and standard (ascorbic acids) were prepared; 25,50,100,150 mg/ml, respectively. Volume was made upto 1000µl with respective solvent used. 1ml of 0.004% of DPPH solution was added to the sample solution of different concentration. 1ml of methanol was added to prepare control instead of plant extract. The blank

for this solution was methanol. As DPPH is sensitive to light, it was exposed to the minimum possible light. These solutions were kept at room temperature in dark for 30 minutes to complete the reaction .

The absorbance was measured at 518 nm and converted into the percentage antioxidant activity using the following equation:

Scavenging capacity (%) = [(Absorbance of control- Absorbance of test)/ Absorbance of control] X 100

The test were done in triplicate. The IC_{50} values were calculated by linear regression of plots. The concentration of sample required to scavenge 50% of DPPH (IC_{50}) were determined.

5) TBARS assay to check Lipid peroxidation :-

Lipid peroxidation was estimated in terms of thiobarbituric acid reactive species (TBARS), using malondialdehyde (MDA) as marker by the method Rani *et al.* with some modifications (Rani *et al*., 2004). 0.5ml of 10% goat brain tissue homogenate was taken in the test tubes. In test sample 0.5 ml of plant extract was added and not in control. Vitamin A and Vitamin E used as standard. 0.1ml of 0.1M aluminum chloride was added to all test tubes and allowed to stand at room temperature for about 3 hrs. 2.0 ml of the TCA-TBA-HCl reagent [(15% (w/v) TCA, 0.375% (w/v) TBA and 0.25N HCl]. The contents were boiled for 15 minutes, cooled and centrifuged at 10,000 g or 7000 rpm for 10 minutes to remove the precipitate. The absorbance was read at 535 nm.

The percent inhibition was calculated from the following formula:

% inhibition= [(Absorbance of control- Absorbance of test)/ Absorbance of control] X 100

RESULT AND DISCUSSION

Phytochemical screening of *S.anacardium* (Table 1) showed the presence of four constituents-glycosides , saponins, tannins and steroid in chloroform extract, four constituents- alkoloid, glycosides, flavonoids and phylobatanins in ethanol extract and five constituents- alkaloids, glycosides, Saponins, flavanoids and phylobataninsin aqueous extract. The amount of total phenolics was found to be highest in ethanol extract (0.507 mgGAE/gm) and lowest in chloroform extract (0.0705mgGAE/gm) (Fig. 1). Whereas the amount of total flavonoid was also found to be variable in all three extract (Fig. 2). Total flavonoid content was highest in aqueous extract (1.042 mgQE/gm) and least in ethanol extract (0.0904mgQE/gm).

Table 1. Phytochemical screening of *S.ancardium* extracts

Extract	Alkoloids	Glycosides	Saponins	Tannins	Flavonoids	Phylobatanins	Steroid
Chloroform	-	+	+	+	-	-	+
Ethanol	+	+	-	-	+	+	-
Aqueous	+	+	+	-	+	+	-

Figure 1. Total Phenolic content

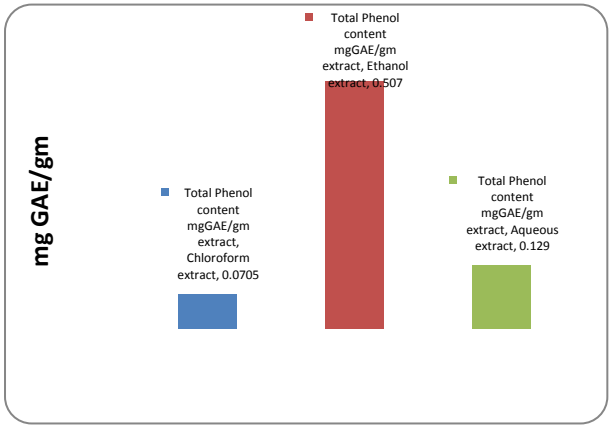
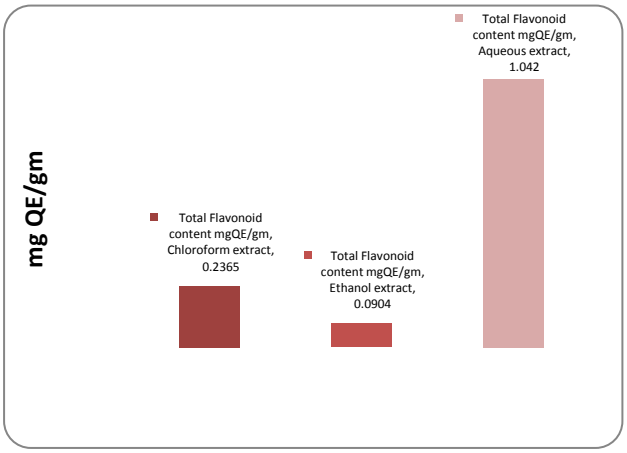


Figure 2. Total Flavonoid Content



In *S. anacardium* antioxidant activity was determined by DPPH assay and expressed as IC_{50} (Fig.3) the concentration at which fifty percent inhibition of free radicals was observed. IC_{50} value of chloroform extract (21.27 µg/ml) was comparable with that of standard Ascorbic acid (28.19 µg/ml). In ethanol & aqueous extract IC_{50} values were 62.24 µg/ml and 137.25 µg/ml respectively, which are very higher than standard Ascorbic acid. The percent inhibition of lipid peroxidation in brain tissue homogenate (Fig.4) was highest in ethanol extract (30.54%) and least in aqueous extract (16.55%). In standard Vitamin A and Vitamin E it was 65.57% and 40.98%

Figure 3. IC_{50} values by DPPH assay

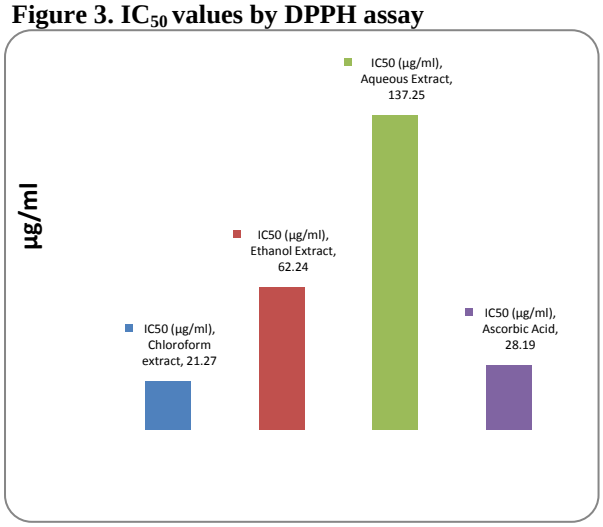
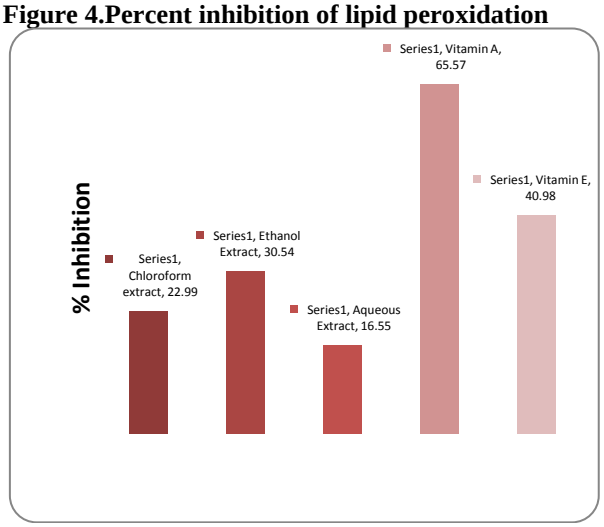


Figure 4.Percent inhibition of lipid peroxidation



respectively. Inhibition of lipid peroxidation is almost similar by ethanol extract and Vitamine E. These results indicated that plant extracts were able to reduce the lipid peroxidation in brain tissue homogenate. From this result it can be concluded that higher phenolic compounds present in ethanolic extracts might be responsible for the higher inhibition of lipid peroxidation so these extracts could be used to alleviate the diseases caused due to the oxidative stress.

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