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Research Article

FREE RADICAL SCAVENGING CAPACITY AND ANTIOXIDANT ACTIVITY OF YOUNG LEAVES AND BARKS OF *ACACIA NILOTICA* (L.) DEL

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ABSTRACT

There has been growing interest in the beneficial health effects of consuming fruits and vegetables. Mainly, the presence of phenolic antioxidants is believed to have the protective mechanisms. In the present study the young leaves and barks of *Acacia nilotica* (L.) DEL. The total phenolic content of the extracts was determined by Folin–Ciocalteu method and antioxidant activity of the extracts was assayed through some in vitro models such as antioxidant capacity by β-carotene-linoleate model system, and radical scavenging activity using α,α-diphenyl-β-picrylhydrazyl (DPPH) method. Recovery percent and total phenolic content of aqueous acetone extracts registered highest values. Though all the extracts exhibited reducing activity, the acetone extracts of both leaf and bark found to be registered highest activity and comparable to that of tannic acid. Like reducing power activity, the similar line of dose dependent trend has also been maintained in the DPPH radical scavenging activity. This might be due to the presence of high molecular compounds like tannins. The results indicated that the extent of antioxidant activity of the extract is in accordance with the amount of total phenolics present in that extract and the young leaves and barks of *Acacia nilotica* is rich in phenolics may provide a good source of antioxidant.

Keywords: *Acacia nilotica*, Total phenolics, Antioxidant activity, DPPH

INTRODUCTION

The role of free radicals and active oxygen in the pathogenesis of human diseases including cancer, aging and atherosclerosis has been recognized [1]. Electron acceptors, such as molecular oxygen, react rapidly with free radicals to become radicals themselves, also referred to as reactive oxygen species (ROS). The ROS include superoxide anions ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\cdot}OH^-$) [2]. Lipid peroxidation, which involves a series of free radical mediated chain reaction processes, is also associated with several types of biological damage. Therefore much attention has been focused on the use of antioxidants, especially natural antioxidants to inhibit peroxidation and to protect from damage due to free radicals.

The medicinal properties of plants have been investigated, in the light of recent scientific developments, through out the world due to their potent pharmacological activities and economic viability. A great number of aromatic, spicy, medicinal and other plants contain chemical compounds, exhibiting antioxidant properties. Source of natural antioxidants are primarily, plant phenolics that may occur in all parts of plants such as fruits, vegetables, nuts, seeds, leaves, roots and barks[3]. Many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, anticarcinogenic,, antibacterial or antiviral activities to a greater or lesser extent[4]. Crude extracts of fruits, herbs, vegetables, cereals and other plant materials rich in phenolic are increasingly of interest in the food industry, because they retard oxidative degradation of lipids and thereby improve the quality and nutritive value of food [5, 6].

Aerobic organisms are continually subjected to reactive oxygen species (ROS), the derivatives of oxygen generated as by-products during cellular metabolism and other exogenous environmental factors such as UV light, ozone, tobacco smoke, different xenobiotics, ionizing radiation herbicides, and pesticides[7]. Oxidative stress, a result of imbalance between the antioxidant defence system and the formation of ROS, may induce damage to cellular biomolecules such as DNA, RNA, proteins, enzymes, carbohydrates, and lipids through oxidative modification and contributing to the pathogenesis of human diseases[8]. As a result, ROS have been implicated in many diseases, including acquired immunodeficiency syndrome, malaria, cardiovascular disease, gastric ulcer, diabetes, malignant tumors, rheumatic joint inflammation, cataracts, Parkinson's and Alzheimer's disease, etc.[9]. Living systems have specific pathways to overcome these repair mechanisms and fail to keep pace with such deleterious effects.

Natural antioxidants such as flavonoids, phenolics, tannins, curcumin and terpenoids are found in various plants[8]. They can reduce the access of oxidants and other deleterious molecules due to their ability to scavenge oxygen-nitrogen-derived free radicals by donating hydrogen atom or an electron, chelating metal catalysts, activating antioxidant enzymes, and inhibiting oxidases[10]. Based on accumulative evidence, in recent decades, tremendous interest has considerably increased in finding natural substances (i.e. antioxidants) present in foods or medicinal plants to replace synthetic antioxidants, which are being restricted due to their side effects. On the other hand, polyphenols, used as natural antioxidants, are gaining importance, due to their health benefits for humans, decreasing the risk of cardiovascular and degenerative diseases by reduction of oxidative stress and counteraction of macromolecular oxidation. Natural antioxidants are also in high demand for application as nutraceuticals/functional food/bio-pharmaceuticals because of consumer preferences.

Acacia nilotica (L.) Del. belongs to the family Mimosaceae and is widely distributed in tropical and subtropical countries. Ayurvedic medicine practices use of natural medicinal plants to promote self healing, good health and longevity, and have declared that *A. nilotica* can provide the nutrients and therapeutic ingredients to prevent, mitigate or treat many diseases or conditions[12]. According to traditionally the bark, leaves, pods and flowers are used against cancer, cold, congestion, cough, diarrhea, dysentery, fever, gall bladder, hemorrhoid, ophthalmia, sclerosis, small pox, tuberculosis, leprosy, bleeding piles, leucoderma and menstrual problems[13]. This plant offers variety of bioactive components such as gallic acid, ellagic acid, isoquercitin, leucocyanadin, kaempferol-7-diglucoside, naringenin- 7-0-b-D-(60-0-galloyl) glucopyranoside, rutin, derivatives of (+)-catechin-5-gallate, apigenin-6,8-bis-C-glucopyranoside, m-catechol and their derivatives. They have spasmogenic, vasoconstrictor, anti/-hypertensive, -mutagenic, -carcinogenic, -spasmodic, -inflammatory, -oxidant and -platelet aggregatory properties[14].

MATERIALS AND METHODS

Folin and Ciocalteu's phenol reagent, DPPH $^{\cdot}$ (2,2-diphenyl-1-picrylhydrazyl) methanol, acetone, ethanol, petroleum ether, tannic acid, linoleic acid, absolute ethanol, potassium ferricyanide, sodium carbonate, trichloro acetic acid, ferric chloride, ammonium thiocyanate were procured from Merck, SRL / S.d.fine chem / Sigma, India.

Plant material preparation

Fresh leaves and young stem bark of *Acacia nilotica* (L.) Del. were collected from Coimbatore, Tamil Nadu. The freshly collected plant materials were washed thoroughly in tap water, shade dried at room temperature (25ºC), powdered and used for solvent extraction. The plant samples were successively extracted with petroleum ether (for disposing lipid and pigments), 70% acetone and 50% ethanol using soxhlet apparatus. Each time before extracting with the next solvent, the material was dried in hot air oven at 40C. The solvents were evaporated using a rotary vacuum-evaporator at 50ºC. The extract recovery in different solvents was expressed as percent of the plant sample dry matter. The freeze-dried extracts thus obtained were dissolved in the respective solvents at the concentration of 1mg/ml and used for assessment of antioxidant capacity through various chemical assays.

Determination of total carbohydrate and total free amino acids

Determination of Total Carbohydrate by Anthrone Method Aliquots of each extracts were taken and made up to 1 ml by adding distilled water. For each tube 4 ml of anthrone reagent was added. All the tubes were covered with glass marbles and heated for 8-10 min in a boiling water bath then the tubes were cooled for 5-7 min. The absorbency of green colour product was measured at 630 nm.

Estimation of Total Free Amino acids by Ninhydrin method

Aliquots of each extracts made up to 1 ml by distilled water. To this 1 ml of ninhydrin reagent was added. The tubes were placed in boiling water bath for 20 min. Then the tubes were cooled and 5 ml of diluents was added to each tube. Then the absorbance was measured at 570 nm [15].

Determination of total phenolic contents

The total phenolic content was determined according to the method described [16]. Aliquots of each extracts were taken in test tubes and made up to the volume of 1ml with distilled water. Then 0.5ml of folin-ciocalteu phenol reagent (1:1 with water) and 2.5ml of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as the tannic acid equivalents (TAE).

Reducing power assay

Aliquots of each extracts were taken in test tubes and dissolved in 1 ml of 0.2 M phosphate buffer in a test tube to which was added 5 ml of a 0.1% solution of potassium ferric cyanide[17]. The mixture was incubated 50°C for 20 min. Following this, 5 ml of trichloro acetic acid (10%) (w/v) solution was added and the mixture was then centrifuged at 7000 rpm for 10 min. A 5 ml of aliquot of the upper layer was combined with 5 ml of distilled water and 1 ml of ferric chloride solution (0.1%) and Absorbance was recorded at 700 nm against reagent blank. A higher absorbance of the reaction mixture indicates greater reducing power of the sample.

Free radical scavenging activity on DPPH•

The antioxidant activity of the extracts was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH**,** according to the method of [18]. Sample extracts at various concentrations was taken and the volume was adjusted to 100µl with methanol. 5ml of a 0.1mM methanolic solution of DPPH was added and shaken vigorously. The tubes were allowed to stand for 20 min at 27C. The absorbance of the sample was measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula:

% DPPH radical scavenging activity = (Control OD – Sample OD / Control OD) X 100.

Inhibition of lipid oxidation in linoleic acid emulsion

Aliquots of each extracts were taken in test tube 0.5 ml of absolute ethanol were added with 0.5 ml of linoleic acid (2.5%) in 99.5% ethanol and 1 ml of 0.05 M phosphate buffer (pH7.0) and 0.5 ml of distilled water was placed in a screw capped tube and then in dark oven at 40°C. A control without sample extracts was used. Every (12 hrs) 0.1 ml aliquot of the solution was taken and 9.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate were added. After exactly 3 min 0.1 ml of 2 x 10^{-2}M ferrous chloride in 3.5% hydrochloric acid was added. The reading was taken at 500 nm until the absorbance of the control reached maximum[19].

AA=100 [(Sample OD at 48hrs –Sample OD at 0h) / (Control OD at 48 hrs - Control OD at 0 h)] X100

STATISTICAL ANALYSIS

Results were expressed as the means of three replicates ± the standard deviation of triplicate analysis.

RESULTS AND DISCUSSION

Total soluble carbohydrates and total free amino acids

Recovery per cent of extract, total soluble carbohydrates and total free amino acids of different solvent extracts of young leaves and young stem bark of *Acacia nilotica* are given in Table 1. 70% acetone extracts of both leaf and bark samples show the higher recovery percent (38.5% and 43.5%, respectively) than the 50% ethanol extracts (2.8% and 2.3%, respectively). The amount of total soluble sugars present in the aqueous ethanol extracts of leaf found to be much lower (2.41%) than the other three extracts namely, aqueous acetone extract of leaf (7.85%), aqueous ethanol extract of bark (6.11%) and aqueous acetone extract of bark (9.51%). The percentage of total free amino acids of aqueous ethanol extracts of both leaf and bark samples found to be higher (7.09% and 8.07%, respectively) than the aqueous acetone extracts (3.88% and 4.34%, respectively). Similarly[17] have reported high yield potential of different solvent extracts of various parts of Indian laburnum (*Cassia fistula*). The results indicate that the yield of extract is greater with more polar solvents and more over it is more effective in extraction of natural antioxidants [20, 21].

Table 1: Recovery per cent of extracts, total soluble carbohydrates and total free amino acids of different solvent extracts of leaf and bark of *A. nilotica* g/100g

Parameter	Leaf		Bark	
Extracts	50% Ethanol	70% Acetone	50% Ethanol	70% Acetone
Recovery per cent Of extract	2.8	38.5	2.3	43.5
Total soluble carbohydrates	2.41± 0.20	7.85 ± 1.98	6.11 ± 1.30	9.51 ± 1.27
Total free amino acids	7.09 ± 0.39	3.88 ± 0.63	8.07 ± 0.21	4.34 ± 0.68

Total Phenolics

Figure1 shows the total phenolic content of different solvent extracts of leaf and bark samples of *A. nilotica*. It is interesting that aqueous acetone extracts of leaf contained highest amount of phenolic compound (25.80%) followed by aqueous acetone extract of bark (19.25%). However aqueous ethanol extracts of both leaf and bark extracts show lowest concentration of total phenolics (4.55% and 6.83%, respectively). In general the total phenolic compounds in *A. nilotica* reported in this study have higher than that of other reports [22] in certain vegetables and [23] in buck wheat (*Fagopyrum esculentum*) (0.313 - 0.333). When compare to some of the tree species such as *Pinus nigra, Cedrus doedara, Pinus halepensis, Abies equitrojani, Cupressus sempervirens,* and the total phenolic content of acetone extracts from both leaf and bark of *A. nilotica* found to be registered higher values. However the reverse trends have been observed with respect to the aqueous ethanol extract of *A. nilotica* samples. Nonetheless, different levels reported in these studies might be attributed to the different parts of plants and methods used (using different standard phenolics). The major contributors of phenolic compounds in *A. nilotica* are catechin, epicatechin, gallic acid, tannin, chlorogenic acid.

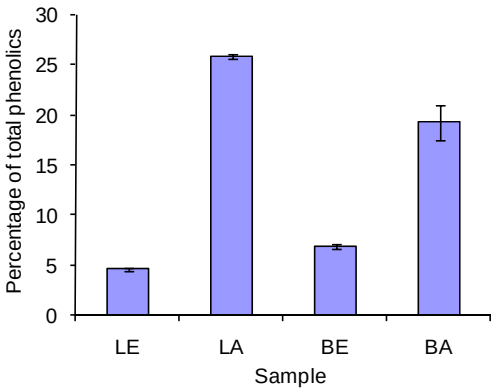

Figure 1: Total phenolic content of different solvent extracts of leaf and bark samples of Acacia nilotica

Antioxidant activity

Reducing power assay

Figure 2-5 show the reducing power of the extracts using potassium ferricyanide reduction method. It has been reported that the reducing power was associated with the antioxidant activity and this relationship of phenolic constituents have been well established in several plant sources including vegetables [16, 24]. The yellow colour of the test solution changes to various shades of green and blue depending upon the reducing power of each extract. The presence of reductants (antioxidants) in the herbal extracts causes the reduction of Fe^{3+}/ ferriccyanide complex to ferrous form. Therefore the Fe^{2+} complex can be monitored by measuring the formation of Perl's Prussian blue at 700 nm[25]. At 0.125 mg dose the reducing power of aqueous acetone extracts of *A. nilotica* leaf and bark found to be far superior to the aqueous ethanol extract of respective samples. In general the increasing absorbance value is directly proportional to the concentration of the extracts. Therefore the increasing OD value implies increasing trend of reducing power. Polyphenolics in the *A. nilotica* leaf and stem bark extracts appear to function as good electron and hydrogen - atom donors and therefore should be able to terminate radical chain reaction by converting free radicals to more stable products. In the present study, the reducing power is found to be in the following order: aqueous acetone extract of bark > aqueous acetone extract of leaf > aqueous ethanol extract of bark > aqueous ethanol extract of leaf. On the other hand a relative low effect on reducing power in ethanol extracts of leaf and bark might be due to the presence of sugars and amino acids, which responsible for acting as prooxidants. Interestingly aqueous acetone extracts of both leaf and bark extracts possess equivalent reducing power in terms of hydrogen donating ability at the concentration of 0.025 - 0.100 mg in the final reaction mixture. Further the values are comparable to that of tannic acid.

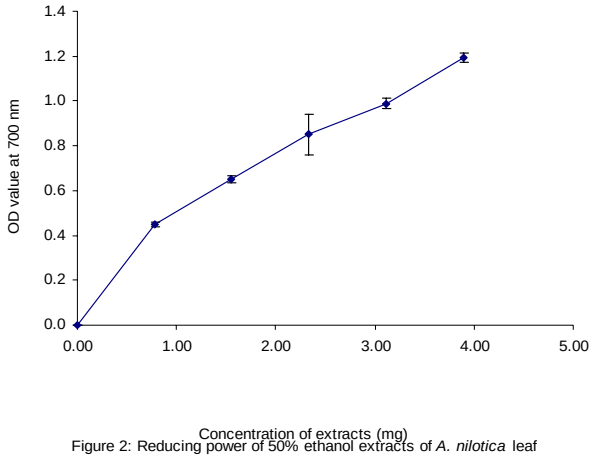

Figure 2: Reducing power of 50% ethanol extracts of *A. nilotica* leaf

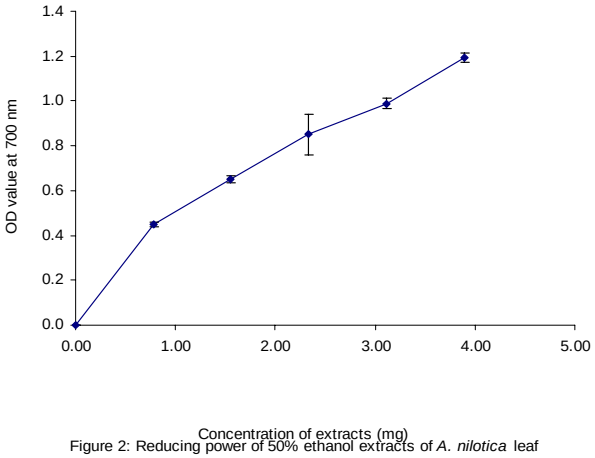

Figure 2: Reducing power of 50% ethanol extracts of *A. nilotica* leaf

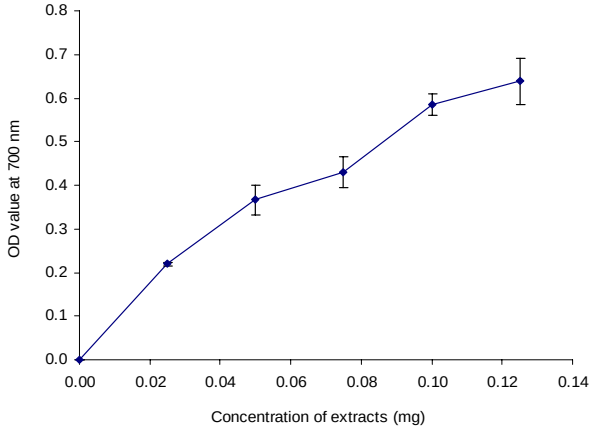

Figure 3: Reducing power of 70% acetone extracts of *A. nilotica*

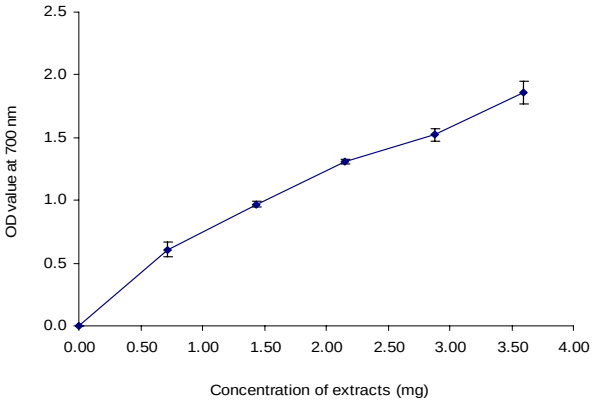

Figure 4: Reducing power of 50% ethanol extracts of *A. nilotica* bark

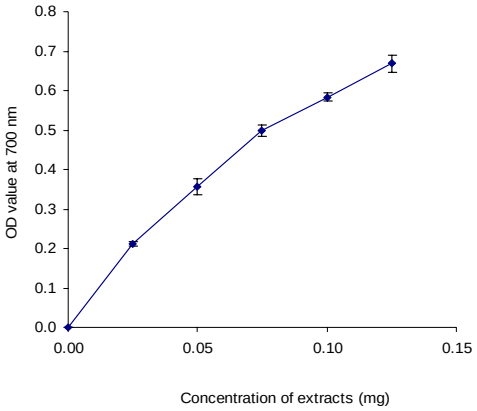

Figure 5: Reducing power of 70% acetone extracts of *A. nilotica* bark

DPPH radical scavenging activity

The radical scavenging activity of the various extracts was tested using methanolic solution of the stable free radical DPPH. Unlike laboratory generated free radical such as the hydroxyl radical and superoxide anion, DPPH • has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition brought about by various additives. A freshly prepared DPPH solution exhibits a deep purple colour generally fades/disappears when an antioxidants present in medium. Thus antioxidant molecule can quench DPPH free radicals (by providing hydrogen atom or by electron transfer, conceivably via a free radical attack on the DPPH molecule) and convert them to a colourless product (2, 2-diphenyl-1-hydrazine, or a substituted analogous hydrazine) resulting in a decreasing absorbance at the 517 nm[26]. Hence the more rapidly the absorbance decreases the more potent antioxidant activity of the extract in terms of hydrogen atom donating capacity/electron transfer ability.

The free radical scavenging activity values of the crude 50% ethanol and 70% acetone extracts from the leaf and bark were examined and compared against one another. Figure 6 -9 reveal that the dose response curve for the free radical scavenging activity of the plant extracts; results are expressed as a percentage of the ratio of the decrease in absorbance at 517 nm to the absorbance of DPPH solution in the absence of phenolic at 517 nm. At a dosage ranging between 0.013 - 0.038 mg of acetone extracts of both leaf and bark offered increasing percentage of free radical scavenging activity (17.3% - 66.7%). The ethanol extracts of leaf and bark contained the Lowest concentration of polyphenolics exhibited the weakest free radical scavenging activity. The data are in agreement with those measured from the reducing power assay, which is to be expected.

Radical scavengers may protect tissues from free radicals, thereby preventing diseases such as cancer[27]. Even though it is unclear whether active constituents in plant extracts, such as those from *A. nilotica* leaf and bark are active against free radicals after being absorbed and metabolized cells in the body, radical scavenging assay were gained acceptance for their capacity to rapidly screen materials of interest.

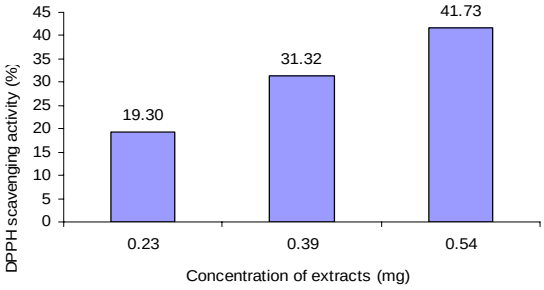

Figure 6: DPPH scavenging activity of 50% ethanol extracts of *A. niloltica* leaf

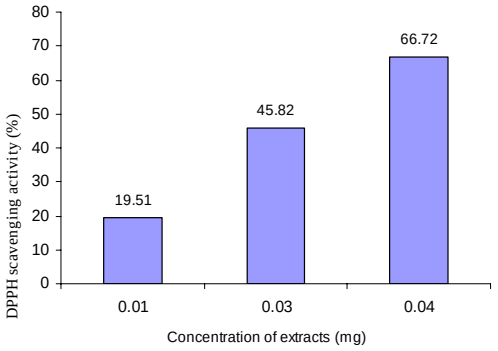

Figure 7: DPPH scavenging activity of 70% acetone extracts of *A.nilotica* leaf

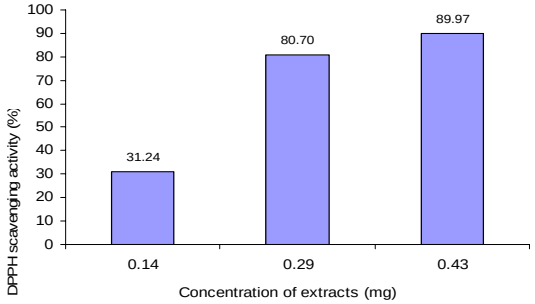

Figure 8: DPPH scavenging activity of 50% ethanol extracts of *A. nilotica* bark

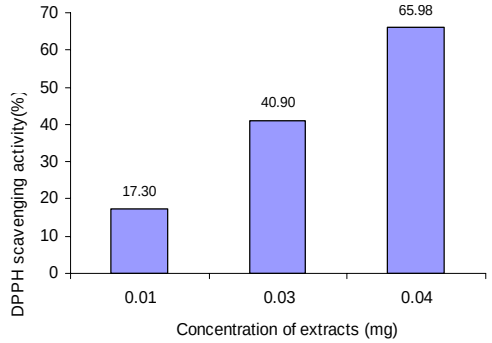

Figure 9: DPPH scavenging activity of 70% acetone extracts of *A.nilotica* bark

Antioxidant activity in linoleic acid emulsion

The results of peroxidation inhibiting activity of aqueous ethanol and aqueous acetone extracts of two different parts of *A. nilotica* in linoleic acid emulsion system are shown in Figure 10. At the concentration of 0.5 mg in the reaction mixture, peroxidation inhibiting percentage of both ethanol and acetone extracts of leaf sample are comparable to each other (25.72% and 25.86%). Whereas acetone extracts of bark (30.39%) is found to be higher than the ethanol extract (21.11%). However the highest antioxidant activity was observed for α-tocopherol in linoleic acid-emulsion

system. Similar results have also been observed in different solvent extracts of leaf and stem bark of *Cassia fistula*[17]. The efficacy of the *A. nilotica* extracts to inhibit oxidation of the linoleic acid-emulsion is a reflection of the complexity of the extract composition (aqueous versus hydrophobic nature of compounds) as well as potential interaction between the extract and emulsion component (oil: water or lipid: air interfaces).

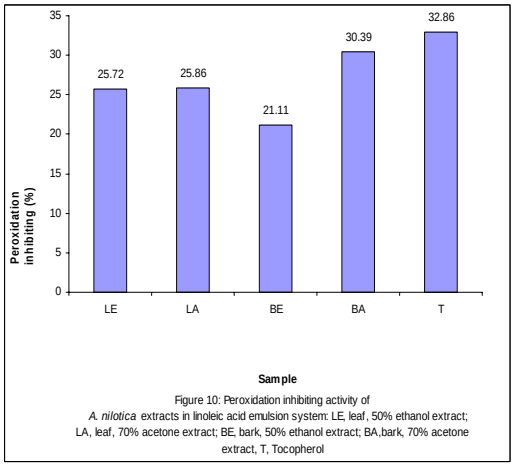

Figure 10: Peroxidation inhibiting activity of *A. nilotica* extracts in linoleic acid emulsion system: LE, leaf , 50% ethanol extract; LA, leaf , 70% acetone extract; BE, bark, 50% ethanol extract; BA,bark, 70% acetone extract, T, Tocopherol

The polar paradox occurs in emulsions the nonpolar compounds have strong antioxidant activity in an emulsion due to the concentration of antioxidant at the lipid: air interface, thereby ensuring strong protection of the emulsion against oxidation. Conversely polar compounds exhibit weak antioxidant activity in emulsion due to the dilution of these compounds in the aqueous phase. Moreover the opposite trend of antioxidant activity profile is observed in bulk lipid or oil system. In the present study the antioxidant activity against the linoleic acid emulsion system might be due to the presence of catechin analogues, tannins and chlorogenic acid.

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