Research Article

Anti-inflammatory and Antioxidant Activities of Polysaccharide from *Adansonia digitata*: an *in vitro* Study

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ABSTRACT

The polysaccharide was obtained from *Adansonia digitata* (AD) and purified by gel permeation chromatography. Results of chemical and HPLC analysis, including one peak showed that was mainly composed of fructose and glucose (8:1), and its molecular weight was about 5.3×10^4Da. The antioxidant effect was determined by many testes including radical scavenging, reducing power, metal chelation, superoxide anion radical scavenging and nitrite scavenging *in-vitro*. The investigation demonstrated that AD showed potential antioxidant activity in a concentration dependent response. AD at 300 and 600 µg/ ml showed 100% effect as chelator for radical, superoxide radical, hydrogen peroxide and metal, therefor, it inhibited lipid peroxidation (100%) and nitric oxide formation (96%). Further, in some case the AD showed the highest antioxidant effect among the standards. The anti-inflammatory activities of polysaccharide at different concentrations were assessed by cyclooxygenase inhibition assay. The ability of the polysaccharide to inhibit cyclooxygenase enzymes (COX-1 and COX-2) was determined by calculating percent inhibition of hydrogen peroxide production. Both enzyme inhibited by polysaccharide and it was more efficient in inhibiting Cox-2 than Cox-1 at all concentrations; therefore, AD could be a potential antioxidant and anti-inflammatory food supplement.

Keywords: Polysaccharide, antioxidant, anti-inflammatory, *Adansonia digitata.*

INTRODUCTION

Edible wild indigenous plants become an alternative source of food with high potential of vitamins, minerals and others interesting elements particularly during seasonal food shortage.[1] Wild fruits are also known to have nutritional and medicinal properties that can be attributed to their antioxidant effects and they can be used to fortify staple foods particularly for malnourished children.[2] The reactive oxygen species (ROS) and free radical-mediated reactions play an important role related to the pathogenesis of various serious diseases, such as neurodegenerative disorders, cancer, cardiovascular diseases, atherosclerosis, cataracts, and inflammation.[3] Antioxidants are substances that delay or prevent the oxidation of cellular oxidizable substrates. They exert their effects by scavenging reactive oxygen species (ROS), activating a battery of detoxifying proteins, or preventing the generation of ROS.[4] In recent years; there has been increasing interest in finding natural antioxidants. It has been accepted that the edible plant with antioxidant properties play important role in human health. The present studies aimed at providing complete proximal and minerals composition of the fruit pulp and seeds of *Adansonia digitata a* (monkey bread) through characterization and quantification of their main phenolic and bioactive compounds. *Adansonia digitata,* called the baobab tree in both Europe and Africa, is belongs to the Malvaceae family.[5,6] The plant is a very massive tree (Figure 1) with a very large trunk which can grow up to 25 m in height and may live for hundreds of years. The plant is widespread throughout the hot and dried regions of tropical Africa.[7] Baobab tree has multi-purpose uses and every part of the plant is reported to be useful.[8,9] The leaves, for instance, are used in the preparation of soup. Seeds are used as a thickening agent in soups, but they can be fermented and used as a flavouring agent, or roasted and eaten as snacks.[10] The bulb is either sucked or made into a drink while the bark is used in making ropes.[8] The different parts of the plant provide food, shelter, clothing and medicine as well as material for hunting and fishing.[11,12]

Baobab leaves, bark, roots, bulb and seeds are used for multiple medicinal purposes in many parts of Africa and were found to show interesting pharmacological properties including antioxidant, prebiotic-like activity, anti-inflammatory, analgesic, antipyretic activity, anti-diarrhoea, anti-dysentery activity and excipient.[13] Baobab seeds, eaten raw or roasted, have a pleasant nutty flavour and are a good coffee substitute, when roasted and ground. The seeds have a very high oil content, tough husk and soft kernel, devoid of starch.

Figure 1: Open fruit with the dried fruit pulp (picture: courtesy of PhytoTrade Africa)

MATERIALS AND METHODS

Chemicals

Ammonium thiocyanate was purchased from E. Merck. Ferrous chloride, polyoxyethylenesorbitan monolaurate (Tween-20), Ascorbic acid (Vc), 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine), nicotinamide adenine dinucleotide (NADH), butylated hydroxytoluene (BHT), and trichloroacetic acid (TCA), Leuco-2,7-dichlorofluorescien diacetate, hematin, arachidonic acid were purchased from Sigma-Aldrich, Germany. Dry *Adansonia digitata* fruit obtained from Dr. Amera Shawky, Department of Natural Resources (DNR), Institute of African Research and Studies, Cairo University.

Methods

Preparation of polysaccharide

Dry *Adansonia digitata* fruit was weighed and mixed with 30 volumes of distilled water at 80 °C for 3 h, in order to remove water-soluble polysaccharide. The water extract was deproteinezed by TCA (5%) and left overnight at 4°C and centrifuged at 5000 rpm for 10 min to remove proteins. The pH of the clear solution was adjusted to 7.0 and dialyzed three times against distilled water (3×1 mL). The dialyzed solution was subjected to fractional precipitation by 1, 2 and 3 volume chilled ethanol and kept overnight at 4°C. The precipitate from ethanol dispersion was collected by centrifugation at 5000 rpm for 2o min under cooling using Sigma-Laborzentrifugen, 2K15). The polysaccharides were washed with acetone, diethyl ether and desiccation in-vacuo.

Analysis of monosaccharide composition

The polysaccharide (0.1 g) was hydrolyzed with 90% formic acid at 100 °C in a sealed tube for 5 h. Excess acid was removed by flash evaporation on a water bath at a temperature of 40 °C and co-distilled with water (3×1 mL).[14] The monosaccharides contents were quantified by HPLC on a Shimadzu Shim-Pack SCR-101N column (7.9 mm × 30 cm), using deionized water as the mobile phase (flow rate 0.5 mL/min), as described by El-Sayed *et al.*[15]

Molecular weight determination

The molecular weight of the polysaccharide was determined by gel permeation chromatography (GPC) on a Sephadex G-200 column (2.4 cm × 90 cm). Dextrans 40, 500 and 2000 kDa (Fluka Chemical Co., Buchs, Switzerland) and glucose, then the elution volumes were plotted against the logarithm of their respective molecular weights. The elution volume of the polysaccharide was plotted in the same graph, and the molecular weight was determined.[16]

Periodate oxidation

The sample (30 mg) dissolved in 12.5 mL of distilled water was mixed with 12.5 mL of 30 mmol/L $NaIO_4$. The solution was kept in the dark at room temperature; 0.1 mL aliquots were withdrawn at 24 h intervals, diluted to 25 mL with distilled water and read in a spectrophotometer at 223 nm[17]. Periodate consumption was calculated on the basis of the change of the absorbance at 223 nm. The solution of periodate product (2mL) was used to assess the amount of formic acid by titration with 0.005 mol/L NaOH.

Viscosity of polysaccharide

The viscosity of the polysaccharide was measured according to Page and Sadoff[18] using a conventional Ostwald Viscometer (Technico, Balu 696) at 30°C.

Apparent relative viscosity (APP) was determined as follows: $\eta_{(app)} = t_s/t_\circ$

Where t_s, is the falling time in the sample at 30°C and $t_\circ$ is that in water under the same conditions.

Infrared Spectroscopy

The Fourier-transform infrared (FTIR) spectrum of the polysaccharide was measured using a Bucker scientific 500-IR Spectrophotometer. The exopolysaccharide was mixed with KBr powder, ground and pressed into a 1 mm pellets for FTIR measurements.[19]

Antioxidant activities

Free radical scavenging activity

Briefly, 0.1 mM solution of $DPPH^{\bullet}$ in ethanol was prepared. Then, 1ml of this solution was added to 3ml of AD polysaccharide and standards solution at different concentrations (75, 150, 300 and 600µg/ml). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517nm in a spectrophotometer (Schimadzu UV/Vis-240IPC). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.[20] The $DPPH^{\bullet}$ radical concentration in the reaction medium was calculated from the following equation: $DPPH^{\bullet}$ scavenging effect (%) = 100 – $[(A_0-A_1)/A_0) \times 100]$ Where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of polysaccharide.[21]

Reducing power

The different concentrations of AD polysaccharide (75, 150, 300 and 600 µg/ ml) in 1ml of methanol were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 ml, 1%). The mixture was incubated at 50 °C for 20 min. A portion (2.5 ml) of TCA (10%) was added to the mixture, which was then centrifuged for 10 min at 1000 ×*g* (MSE Mistral 2000, UK, and Serial No.: S693/02/444). The upper layer of solution (2.5 ml) was mixed with methanol (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm in a spectrophotometer[22]. Ascorbic acid and BHT was used as controls. Higher absorbance of the reaction mixture indicated greater reducing power.

Metal chelating activity

Briefly, polysaccharide and standards (75, 150, 300 and 600 µg/ ml) were added to a solution of 2mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml) and the mixture was shaken vigorously and left standing at room temperature for ten minutes. After the mixture had reached equilibrium, the absorbance of the solution was then measured spectrophotometrically at 562 nm in a spectrophotometer.[23] The percentage of inhibition of ferrozine-Fe^{2+} complex formation was given by the formula: % = $[(A_0-A_1)/ A_0] \times 100$. Where A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of polysaccharide and standards. The control contains $FeCl_2$ and ferrozine.[24]

Superoxide anion scavenging activity

Superoxide radicals are generated in phenazine methosulphate (PMS)-nicotinamide adenine dinucleotide (NADH) systems by oxidation of NADH and were assayed by the reduction of nitroblue tetrazolium (NBT). In this experiments, the superoxide radicals were generated in 3 ml of Tris-HCl buffer (16 mM, pH 8.0) containing 1 ml of NBT (50 µM) solution, 1 ml NADH (78 µM) solution and 1ml sample solution of AD polysaccharide at different concentrations were mixed. The reaction was started by adding 1 ml of PMS solution (10 µM) to the mixture. The reaction mixture was incubated at 25ºC for 5 min, and the absorbance at 560 nm in a spectrophotometer was measured against blank samples.[25,26] Ascorbic acid and BHT was used as controls. Decrease in absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula: % = $[(A_0-A_1)/A_0] \times 100$. Where A_0 was the absorbance of the control, and A_1 was the absorbance of polysaccharide or standards.

Scavenging of hydrogen peroxide

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm in a spectrophotometer. Polysaccharide and standards (75, 150, 300 and 600 µg/ ml) in methanol were added to a hydrogen peroxide solution (0.6 ml, 40 mM). Absorbance of hydrogen peroxide at 230 nm was determined after ten minute against a blank solution containing in phosphate buffer without hydrogen peroxide.[27] The percentage of scavenging of hydrogen peroxide of polysaccharide and standard compounds was calculated using the following equation: H_2O_2 (%) = $[(A_0-A_1) A_0] \times 100$. Where A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of polysaccharide and standards.[28]

Nitric Oxide radical scavenging activity

$NO^{\bullet}$ radical scavenging activity of test compounds was determined by using a Sodium nitroprusside (SNP) generating $NO^{\bullet}$ system. $NO^{\bullet}$ generated from SNP in aqueous solution at physiological pH reacts with oxygen to produce nitrite ions which were measured by the Greiss reagent[29] which constitutes1% Sulfanilamide in 5% ortho-H_3PO_4 and 0.1 % Naphthylethylene diamine dihydrochloride. The reaction mixture (2 ml) containing various concentrations of the test polysaccharide and standard compounds and SNP (final concentration, 10 mM) in phosphate buffered saline (PBS) pH 7.4 were incubated at 25°C for 150 min. After incubation, 1ml samples of reaction mixtures were removed and diluted with 1 ml Greiss reagent. The absorbance of these solutions was measured at 540 nm against the corresponding blank solution. Rutin was used as a reference standard.

Total antioxidant activity

Exactly 0.2 ml of peroxidase (4.4 units/ ml), 0.2 ml of H_2O_2 (50 µM), 0.2 ml of ABTS (2,2-azino-bis(3-ethylbenz-thiazoline-6-sulfonic cid, diammonium salt, 100µM) and 1ml methanol were mixed, and were kept in the dark for 1 hour to form a bluish green complex after adding of 1 ml AD polysaccharide of different concentrations or ascorbic acid and BHT, used as a control.[30,31] All were tested in triplicates. The absorbance at 734 nm was measured to represent the total antioxidant activity and then was calculated as follows: Total antioxidant activity (%) = $[1- (A_{sample}/A_{control})] \times 100$.

Lipid Peroxidation-Ammonium Thiocyanate

A pre emulsion was prepared by mixing 175 µg Tween 20, 155 µL linoleic acid, and 0.04 M potassium phosphate buffer (pH 7.0). A 1 mL of sample in 99.5% ethanol was mixed with 4.1 mL linoleic emulsion, 0.02 M phosphate buffer (pH 7.8) and distilled water (pH7.9). The mixed solutions of all samples (21mL) were incubated in screw cap-tubes under dark conditions at 40˚C at certain time intervals. To 0.1 mL of this mixture was pipeted and added with 9.7 mL of 75% and 0.1 mL of 30% ammonium thiocyanate sequentially. After 3 min, 0.1 mL of 0.02 M ferrous chloride in 3.5% HCl was added to the reaction mixture. The peroxide level was determined by reading daily of the absorbance at 500 nm in a spectrophotometer. Antioxidant assay of ascorbic acid and BHT were also determined for comparison.[32] All test data was the average of three replicate analyses. The inhibition of lipid peroxidation in percentage was calculated by the following equation: Inhibition (%) = $[(A_0 - A_1) / A_0] \times 100$, Where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of polysaccharide or standard compounds.

Anti-inflammatory activity

The oxidation of leuco-dichlorofluorescein (1-DCF) in the presence of phenol by the hydroperoxide formed in the cyclooxygenase reaction can be used as a sensitive spectrophotometric assay for PGH synthase activity. Leuco-2,7-dichlorofluorescein diacetate (5 mg) was hydrolysed at room temperature in 1 M NaOH (50 µL) for

10 min, then 1M HCl (30 µL) was added to neutralize excess NaOH before the resulting 1-DCF was diluted in 0.1 M Tris-buffer, pH 8. Cyclooxygenase enzyme (COX-1 or COX-2) was diluted in 0.1 M Tris-buffer, pH 8, so that a known aliquot gave an absorbance change of 0.05/ min in the test reaction. Test samples (or the equivalent volume of methanol, 20 µL) were pre-incubated with enzyme at room temperature for 5 min in the presence of hematin. Premixed phenol, 1-DCF and arachidonic acid were added to the enzyme mixture to begin the reaction, and to give a final reaction mixture of arachidonic acid (50 µM), phenol (500 µM), 1-DCF (20 µM) and hematin (1 µM) in 1 mL final volume of 0.1 M Tris-buffer, pH 8. The reaction was recorded spectrophotometrically over 1 min at 502 nm.[33] A blank reaction mixture was analyzed in the spectrophotometer reference cell against each test reaction to account for any non-enzymatic activity attributed to the test sample. This blank consisted of the reaction mixture without the addition of enzyme. Celecoxib was used as standard compound.

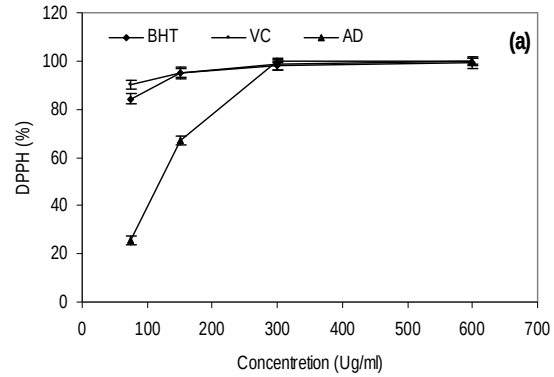
Statistical analysis

Conventional statistical methods were used to calculate means and standard deviations of three replicates were carried out with the different methods. Analysis of variance (ANOVA) was applied followed with Pot Hoc test to determine differences ($p< 0.01$).

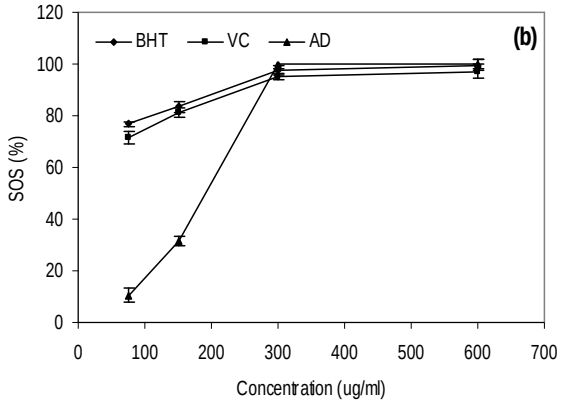
RESULTS

Structural characterization of polysaccharide

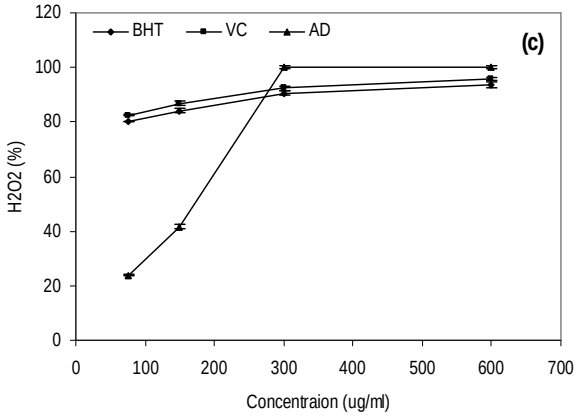
AD polysaccharide was obtained from the fruit bodies of *Adansonia digitata a* through a series of gel permeation chromatography (GPC) showed a single and symmetrical peak on Shephadex G-200, indicating its homogeneity. Its molecular weight (MW) was determined as 5.3×10^4 Da according to the elution volume. The total sugar content of AD polysaccharide was determined to be 98.4% by using the phenol-sulfuric method. HPLC analysis indicated that it was composed of glucose and fructose at molar ratio of 1:8, respectively; and the relative viscosity was 12.56 than water at 30 ºC. The infrared spectrum of AD polysaccharide displayed a broad stretching intense characteristic peak at 3436.53 cm^{-1} were due to the hydroxyl stretching vibration of the polysaccharide. The bands in the region of 2925.48 cm^{-1} were due to C–H stretching vibration, and the bands in the region of 1628.59 cm^{-1} were due to associated water. Moreover, the characteristic absorptions at 915 cm^{-1} in the IR spectra indicated that β-configurations were simultaneously present in polysaccharide. The AD polysaccharide showed abundant periodate uptake, while it was oxidized. The consumption of periodate 0.584 mol was thirty-times more than the amount of formic acid 0.02 mol, that was produced after periodate treatment, indicating the presence of little amounts of monosaccharides, which are (1---4)-linked, and/or (1---2)-linked.



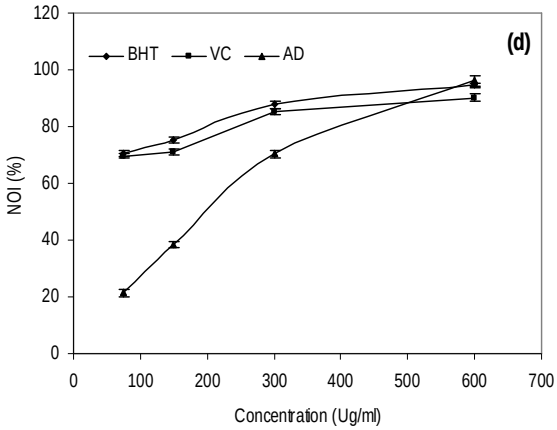
(a) scavenging of DPPH radicals



(b) scavenging of superoxide radicals



(c) inhibitor hydrogen peroxide



(d) inhibition of nitrite formation.

Figure 2: Antioxidant property of the AD-polysaccharide

Values were representative of three separated experiments.

Antioxidant activities

DPPH radical scavenging effect

AD polysaccharide was significantly scavenged the DPPH radicals to reach the maximum inhibition percentage (100%) at 300 and 600 µg/ ml (Figure 2a). AD polysaccharide was most effective in decolorization of reaction mixture than the two tested standards at high concentrations (300 and 600 µg/ ml) while it showed the lowest effect at 75 and 150 µg/ ml, 25.63 and 66.89 %, respectively. The radical scavenging activity was concentration dependent**.**

Superoxide radical scavenging effect

Figure (2b) shows the percentage inhibition of superoxide radical generation by 100% at 300 and 600 µg/ ml of AD polysaccharide and comparison with same doses of BHT and ascorbic acid. The AD polysaccharide exhibited higher superoxide radical scavenging activity than BHT and ascorbic acid ($P < 0.05$) at 300 and 600 µg/ml. The inhibition percentage of superoxide generation by 75 µg/ml amount of AD polysaccharide was found as 10 and 31.4% and smaller than that of some doses BHT, and ascorbic acid (76 and 71% at 75µg/ml and 83 and 81% at 150µg/ml), respectively. Superoxide radical scavenging activity of those samples at 300 and 600 µg/ ml followed the order: AD polysaccharide> BHT > ascorbic acid.

Inhibition of hydrogen peroxide

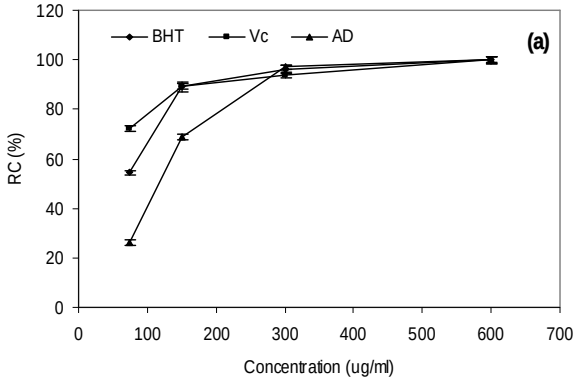
The ability of AD polysaccharide to scavenge H_2O_2 was determined and demonstrated in Figure (2c) as compared with BHT and ascorbic acid. AD polysaccharide was capable of scavenging H_2O_2 in a dose-dependent manner. 300 and 600 µg/ ml of AD polysaccharide exhibited 100 % scavenging activity on H_2O_2. On the other hand, BHT showed 90.6 and 93.53 % while ascorbic acid produced 92.4 and 95.82% for the same concentrations, respectively. These results indicated that AD polysaccharide possess effective H_2O_2 scavenging activity more than BHT and ascorbic acid. However, there was statistically a very significant correlation between those values and control ($P < 0.01$).

Inhibition of nitrite formation

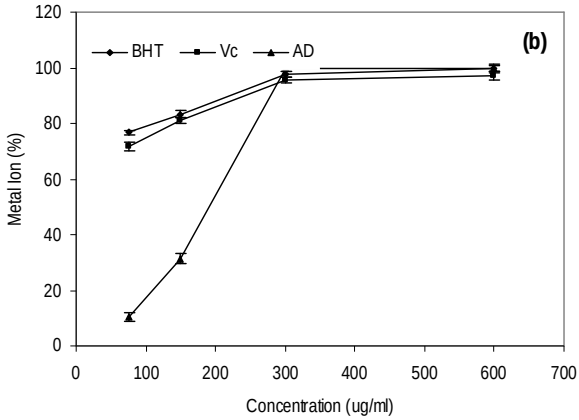
Nitric oxide radical generated by sodium nitroprusside at physiological pH was found to be inhibited by AD polysaccharide at tested concentrations in a dose dependent manner, the highest concentration give the highest inhibition percentage. AD polysaccharide inhibited nitrite production by 96.2 % at 600 µg/ ml which more than BHT by 1.7% and more than ascorbic by 4%**.** BHT and ascorbic acid possess potent effect more than AD polysaccharide at the other concentrations, 75, 150 and 300µg/ml (Figure 2d).

Reduction capability

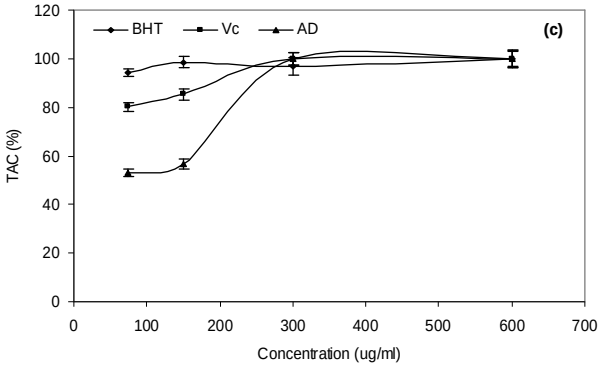
Figure (3a) shows the reductive capabilities of AD polysaccharide compared to standards by $Fe^{3+} \rightarrow Fe^{2+}$ transformation in the presence of AD polysaccharide. AD polysaccharide produced lower reduction capability as compared to tested standards and the effect of polysaccharide was increased dependently with increasing concentration.



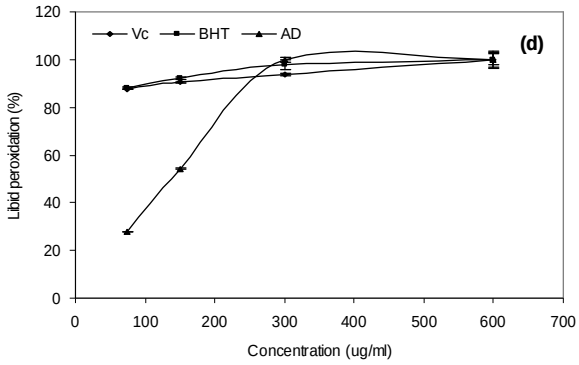
(a) reduction capability



(b) metal ion chelation



(c) total antioxidant activity



(d) inhibition of lipid peroxidation.

Figure 3: Antioxidant property of the AD-polysaccharide

Values were representative of three separated experiments.

Metal ion chelation

As shown in Figure (3b) the formation of the Fe^{2+}-ferrozine complex was not completed in the presence of AD polysaccharide at different concentrations, indicating that AD polysaccharide chelates the iron. Chelation of ions was significantly enhanced with increasing concentration to reach the maximum chelation effect (100%) at 600µg/ml which was the same of two standards. While the lowest effect (26.25%) was recorded at 75µg/ml and it was the least effect as compared to standards.

Total antioxidant activity

AD polysaccharide exerted nearly 100% total antioxidant activity at a level of 300 µg/ ml and it was equal 100% activity at 600 µg/ ml (Figure 3c). Total antioxidant activity was significantly ($p < 0.01$) and gradually increased with increasing concentration; 53, 57, 96.8 and 100% activity for 75, 150, 300 and 600 µg/ ml, respectively.

Inhibition of lipid peroxidation

Lipid peroxidation inhibition effect of AD polysaccharide was determined by the thiocyanate method. AD polysaccharide exhibited effective antioxidant activity at all doses (Figure 3d). The antioxidant activity of AD polysaccharide increased concentration dependently. AD polysaccharide (300 and 600 µg/ ml) showed higher antioxidant (100%) activities than that of BHT and ascorbic acid at the same concentrations (98 and 100% for BHT as well as 93 and 100% for ascorbic, respectively).

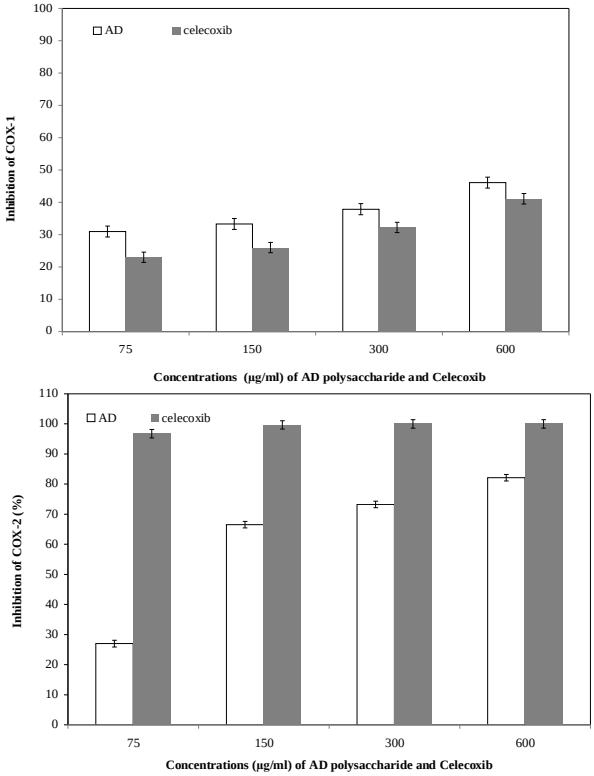


Figure 4: Inhibitory activity of AD polysaccharide and standard (celecoxib) at different concentrations on hydrogen peroxide production formed in oxidation reaction of leuco-dichlorofluorescein by COX-1 (a) and COX-2 (b).

Anti-inflammatory Activity

The present work was carried out to evaluate the inhibitory effect of AD polysaccharide on COX-1 and COX-2. COX-2 was inhibited moderately by AD polysaccharide at different concentrations (75,150, 300 and 600 µg/ ml). Inhibition of COX-2 was concentration dependent. The inhibition was magnified by increasing concentration from 75 to 600 µg/ ml. It inhibited H_2O_2 production in oxidation reaction of leuco- 2, 7-dichlorofluorescein by 26.98, 66.51, 73.22 and 82.11% at 75,150, 300 and 600 µg/ ml, respectively. On the other hand, the standard celecoxib inhibited COX-2 by 97, 99, 100 and 100% for the same concentration, respectively (Figure 4). COX-1 was inhibited significantly by AD polysaccharide and celecoxib but AD polysaccharide found to have higher inhibition effect on COX-1 than celecoxib (Figure 4). It produced 31, 33, 37, 46% inhibition percentage for 75, 150, 300 and 600 µg/ml, respectively, while celecoxib produced 23, 26, 33 and 41% for the same concentration, respectively. That indicate AD polysaccharide has selectivity effect on cyclooxigenase enzymes, it found to have nearly the same effect of celecoxib, selective Non-steroidal anti-inflammatory drug.

DISCUSSION

A sequential extraction with boiling water and ethanol was performed in order to obtain extracts with high molecular weight compounds, such as polysaccharides and low molecular weight compounds, such as phenolic compounds. Both kinds of compounds play important roles in plant, including medicinal functions.[34] The fact that the amount of periodate consumption was more than the amount of formic acid demonstrated other linkages oxidized by periodate, such as (1---4) or (1---2) and not present (1---6)-Linked.[35,36] IR results were confirmed by Bao *et al.*[37] and Park[38]. The biological activities of polysaccharides depended on chemical structural and physical properties such as molecular weight, molar ratio, glycosidic linkage. The antioxidant activity of putative antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging.[39,22] Numerous antioxidant methods and modifications have been proposed to evaluate antioxidant activity and to explain how antioxidants function. Of these, total antioxidant activity, reducing power, DPPH assay, metal chelating, active oxygen species such as H_2O_2, $O_2^{\bullet-}$ and $OH^{\bullet}$ quenching assays are most commonly used for the evaluation of antioxidant activities of extracts.[40,41,42] The scavenging ability of the polysaccharide As 1-1 produced by the mangrove endophytic fungus *Aspergillus* sp.Y16 appears to higher than that of polysaccharides produced by other marine fungi and bacteria.[43-46] In addition, Vaz *et al.*[47] reported that the water soluble polysaccharide fraction from *Clitocybe odora* showed the best antioxidant properties, among the polysaccharide extracts. The inhibition of nitric

oxide (NO•) and cyclooxygenase-2 (COX-2) production is considered to be a promising approach to the treatment of various diseases including inflammation and cancer; therefore, AD polysaccharide may be a useful anti-inflammatory and anticancer agent. The modern theories on the anti-inflaminatory effect of polysaccharides extracted from marine algae have been analyzed .It has been found that polysaccharide play a double role in the immune response as either an initiator or inhibitor of the inflammation process. The polysaccharide may be used as a basis for the immune biological remedies of the new generation to cure allergies, autoimmune diseases and other inflammations of different origins that are accompanied by the hyper production of inflammatory cytokines, nitrogen monoxide, and complements.[48] The polysaccharides from *Pleurotus* species have immunomodulating, hypoglycemic, hypolpididemic, anti-inflammatory, and antitumor activities.[49,50] Cyclooxygenase (COX) are lipid metabolizing enzymes that catalyze the oxygenation of polyunsaturated fatty acids (PUFA), preferably arachidonic acid (AA), to form the prostanoids, which are potent cell-signaling molecules associated with the initiation, maintenance and resolution of inflammatory processes.[51] Selective modulation of the many prostanoids has important therapeutic potential for the treatment of inflammation and inflammatory conditions such as rheumatoid arthritis. Traditional drug treatments for relieving the pain and swelling of inflammation include aspirin, indomethacin and other non-steroidal anti-inflammatory drugs (NSAIDs). Unfortunately, NSAIDs are also noted for undesirable gastrointestinal side effects related to their use.[52] For this reason, selective COX-2 inhibitors (the Coxibs') have come to attention in recent years. As coxibs are increasingly used in clinical practice, it appears that they too exhibit side effects, most notably in relation to cardiovascular disturbances. As a result, medical researchers are looking for safer, more efficacious alternatives to both the traditional NSAIDs and the more recent COX-2 selective inhibitors. AD polysaccharide showed potent inhibitory effect against COX-1 and COX-2 and it was found to have selective effect against COX-2 as compared to celecoxib, selective NSAIDs. AD polysaccharide has promising and selective effect as anti-inflammatory agent that may useful in treating inflammation or related disease. It was reported that oxidative stress, which occurs when free radical formation exceeds the body's ability to protect itself, forms the biological basis of chronic conditions such as arteriosclerosis.[53] Based on the data obtained from this study, AD polysaccharide exhibits free radical inhibitor or scavenger activity for DPPH and superoxide anion radical as well as a primary antioxidant that reacts with free radicals, which may limit free radical damage occurring in the human body. Hydrogen peroxide itself is not very reactive, but it may be toxic to cell since it may give rise to hydroxyl radicals in cells.[4] AD polysaccharide possesses scavenger effect not only for radicals but also for hydrogen peroxide at all concentrations and showed to have the most effect at the maximum concentration (600µg/ml). Also tested AD polysaccharide showed promising effect in inhibiting nitrite formation, this effect was concentration dependent. It produced 96% inhibition percentage for AD polysaccharide at 600µg/ml. Iron can stimulate lipid peroxidation by the Fenton reaction, and also accelerates peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation.[42,54] Data presented in this work showed the potent effect of AD polysaccharide as ferrous ion chelator at all tested concentration and its effect found to be concentration dependent.

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