



Ferric reducing capacity and Antioxidant activity of Ayabirungaraja karpam (ABK)

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Abstract

ABK is the metallic based herbal medicine, no scientific testimonial exist to conform its action and potential efficacy to proof the ancient siddhars' quotation about Ayabirunkaraja katpam which is mentioned in reputed siddha text siddha vaithiya thirattu" whereas quoted it is best remedy for Anemia, grey hair and Weakness.

Powdered samples (10 g) were extracted with 100 ml of solvents (hexane, ethyl acetate, methanol, ethanol and water) and kept for 3 h at room temperature. For antioxidant activity, the solvent was evaporated using a rotovapor (Make: Buchi, Model: R-300) and dry extract was obtained. The extract was then re-dissolved in water at 100 mg / ml ratio and used for the analysis. RBC membrane stabilization potential of plant extracts were investigated according to the methods proposed by Sakat et al. (2010). Human blood (2 ml) was drawn from volunteer in a heparinised tube and centrifuged at 2000 rpm for 10 min. The pellet (RBC cells) was washed twice with PBS (9 ml) and finally the pellet was re-suspended in 10 ml of PBS. The extract (500 µl) were added to 1 PBS, 1 ml of 3% H₂O₂ and incubated for 30 min. Based on the absorbance, the percentage of RBC membrane damage and inhibition of membrane damage were calculated.

It is concluded that the aqueous extract of Auabirungaraja karpam showed appreciable antioxidant and ferric reducing capacities and effective in preventing RBC membrane damage which due to oxidative stress.

Keywords: RBC, Antioxidant, Membrane.

Introduction

ABK is used by Traditional practioners in Sri Lanka and commonly used in government sector and Private Practioners in India. The ABK is included in the essential drug list of siddha medicine by department of AYUSH drug control cell since 2013. The researchers reported that all kayakalpa plants have anti-radical and scavenging activity. The mechanism of antioxidant activity is

describes as, prevention of free radical chain initiation, binding of transition metal iron catalysts, decompositions of peroxides. Prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Diplock et al, 1997). The reducing properties are generally associated with the presence of reductones (Duh,1998),thus exert antioxidant actions by breaking the antioxidant action by breaking the

free radical chain and by donating the hydrogen atom and also reductones reputed to react with certain precursors of peroxide, thus preventing peroxide formation.(Gordon,1990).

Free radical is molecule or molecular fragment that contains one or more unpaired electron in its outermost orbital, highly reactive and imbalance molecules which are by product of normal metabolism and associated with degenerative and destructive process of healthy cells. Free radicals steal electron from healthy cells to neutralize their own charge causing cellular damage because they are like robbers which are deficient in energy. The modern lifestyle, fast foods, obesity and physical inactivity are the major risk factor for production of free radicals and reactive oxygen spp. The generation of free radicals beyond the scavenging ability of antioxidant defences result in Macro and micro vascular dysfunction. Oxidative stress is the prime reason for molecular pathogenesis which result from an imbalance between radical generation and radical scavenging system. The harmful elements are super oxide anions radicals, hydroxyl radicals and hydrogen peroxide .Damaging essential biomolecules such as protein, DNA and lipids which eventually causes inflammation, Kidney diseases, diabetic mellitus, graying, ageing and several degenerative diseases in humans, (Halliwell, 1997). The phenolic acids, Polyphenol, flavinoids inhibit the oxidative stress mechanism and thus prevent damage of cells.

RBC used as model cell by a several researchers to the study of interaction of drugs with membrane. It also has been reported that the production free radicals such as lipid peroxide and superoxide in various condition like heat induced stress haemolysis due to cell membrane de stabilization. (Agarwal and Rangari,2003). The therapist search natural antioxidants since they are free from health hazards. The Ayabirungaraja karpam contain iron compounds and two herbs which are being used in the processing of drug formulation have sound antioxidant activity reported from recent research records.

Therefore the aim and objective of the present study was to determine the phenolic content and to establish the capacity of ferric reducing power, characterize the antioxidant and RBC membrane stabilization assay of the different extract of Ayabirunkaraja karpam.

Methodology

Sample collection: The samples (ABK formulation) were collected from the customer in powdered form by CARISM office (SR No. 17-03-X-2595 dt. 16-03-2017).

Preparation of extract: Powdered sample (10 g) was extracted with 100 ml of solvents (hexane, ethyl acetate, methanol, ethanol and water) and kept for 3 h at room temperature. The extracts were then separated using Whatman No. 1 filter paper and used for further experiments. For antioxidant activity, the solvents were evaporated using a rotovapor (Make: Buchi, Model: R-300) and dry extract was obtained. The extract was then re-dissolved in water at 100 mg / mL ratio and used for the analysis.

Total phenol content: The total phenolic content of extract was estimated according to the method of Singleton et al. (1999). Suitably diluted sample (100 μ l) was taken with 250 μ l of Folin's-Ciocalteu reagent and 1000 μ l of 5% of Na₂CO₃ was added and incubated for 30 min in dark. Then the absorbance was measured at 720 nm using Spectrophotometer. A calibration curve was prepared using standard gallic acid (16 – 100 mg/L; $y = 0.0094x - 0.0585$; $R^2 = 0.9939$) and used to calculate the total phenolic content of the extract and the results were expressed as gallic acid equivalents (mg GAE / 100 g sample).

Ferric reducing power: The reducing power of extract was determined according to the method of Oyaizu (1986). Samples (2.5 ml) in phosphate buffer (2.5 ml, 0.2 M, pH 6.6) were added to potassium ferricyanide (2.5 ml, 1.0%) and the mixture was incubated at 50°C for 20 min.

Trichloroacetic acid (2.5 ml, 10%) was added, and the mixture was centrifuged at 650 x g for 10 min. The supernatant (5.0 ml) was mixed with ferric chloride (5.0 ml, 0.1%), and then the absorbance was read spectrophotometrically at 700 nm. Based on the absorbency value, the ferric reducing power of extract was expressed.

Antioxidant activity: The DPPH radical scavenging assay was used to analyze the antioxidant property of aqueous extract of the sample by following Sanchez-Moreno *et al.* (1998) method. The extract (100 μ l) was added to 0.9 ml of methanolic solution of DPPH (2.5 mg/100 ml) and the reactants were incubated at room temperature for 30 min in dark. Different concentrations of Butylated hydroxyanisol (BHA) were used as a standard and the solvent (distilled water) was used instead of extract in control. After 30 min, the absorbance was measured at 515 nm using a spectrophotometer and the radical scavenging activity of the extract was calculated and expressed on percentage basis.

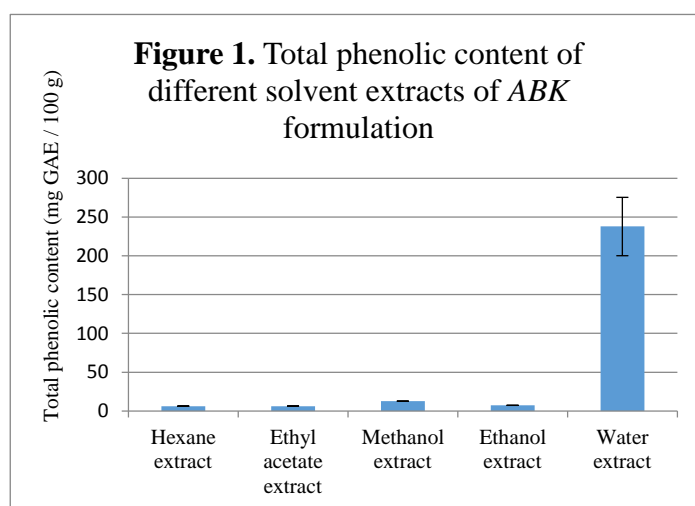
Membrane stabilization potential: RBC membrane stabilization potential of plant extracts were investigated according to the methods proposed by Sakat *et al.* (2010). Human blood (2 ml) was drawn from volunteer in a heparinised tube and centrifuged at 2000 rpm for 10 min. The pellet (RBC cells) was washed twice with PBS (9 ml) and finally the pellet was re-suspended in 10 ml of PBS. The extract (500 μ l) were added to 1 PBS, 1 ml of 3% H₂O₂ and incubated for 30 min. In normal control, 1 ml PBS was added instead of extract and in standard group, 1 ml of ascorbic acid was added instead of extract and in negative control only H₂O₂ was added. After incubation, the contents were centrifuged at 2000 rpm for 10 min and the supernatant was used to measure the absorbance at 520 nm. Based on the absorbance, the percentage of RBC membrane damage and inhibition of membrane damage were calculated.

Extract yield: The extract yield juice of lemon and *Wedelia chinensis* was determined based on Joshi and Aeri (2009) method. Fresh lemon fruits (4 Nos.) were used to prepare the juice by hand squeezing and the *W. chinensis* plant juice was prepared by taking 25 g of fresh material with 50 ml of distilled water and crushed in a domestic mixer grinder for 5 min. Then the contents were filtered in a filter paper and 10 ml volume was transferred to pre-weighed beakers and kept at 105°C for 3 h in a oven. After cooling to room temperature in a desiccator, the gross weight was recorded and based on difference in weight, the extract yield was calculated and expressed on percentage basis.

Results and discussion

Total phenolic content

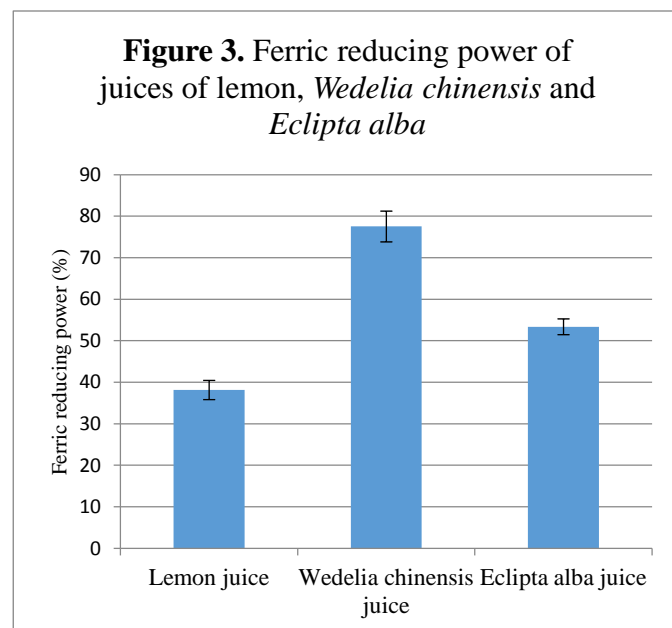
Aqueous extract of ABK formulation was noted to contain higher amount of total phenolic compounds (237 mg GAE / 100 g) when compared to other solvent extracts (Figure 1). Since it is a metal based preparation and the percentage of herbal ingredients are very less, the polyphenolic content was low. The polyphenols present in ABK formulation might be high polar in nature and thus extracted only with water.



Ferric reducing power

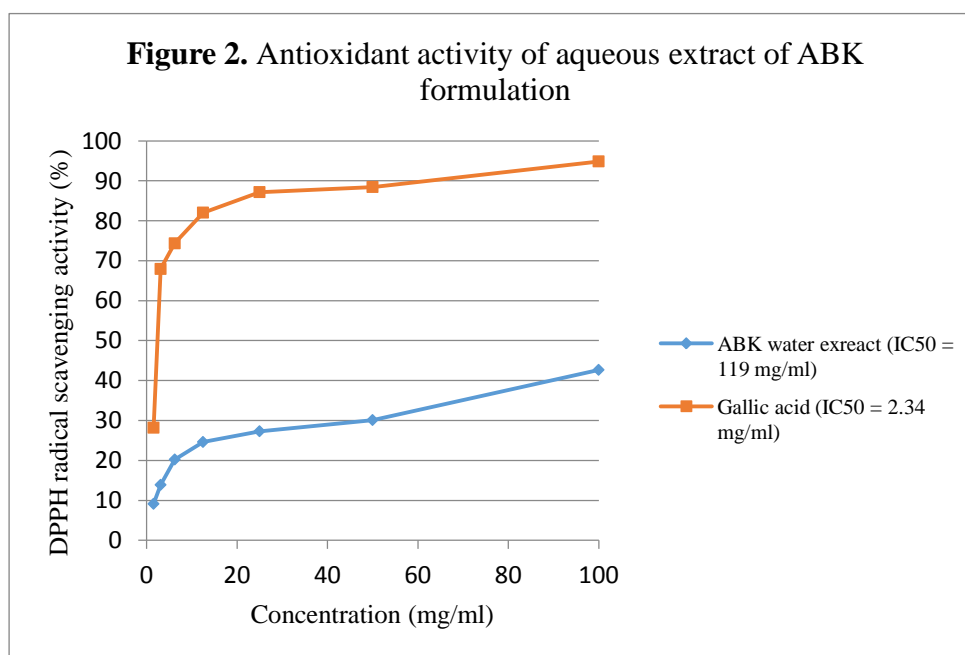
Ferric reducing power of fresh juices of lemon, *Eclipta alba* and *Wedelia chinensis* was analyzing and the results are shown in Figure 3. In this assay, Fe (III) is reduced to Fe (II) by the

antioxidant compound through electron transfer. The reduced Fe (II) forms the Pearl's blue complex, which can be measured at 700 nm. Between the samples analyzed, *W. chinensis* juice exhibited higher ferric reducing power (77.54%) when compared to *Eclipta alba* (53.38%) lemon juice (38.16%). The ferric reducing power of *Wedelia chinensis* juice was found to be higher when compared to *Eclipta alba* juice and this might be the reason why the *Wedelia chinensis* was used to prepare ABK formulation. Since, both lemon and *Wedelia chinensis* juices have remarkable ferric reducing power, the phytochemicals particularly the polyphenols of these plants were utilized for the conversion of ferric form of iron to ferrous form and hence, the final formulation of ABK revealed poor antioxidant power. Conversion of Fe (III) in to Fe (II) form is of great biological significance, because the ferrous form (Fe II) is the only bio-available form and hence, the iron must be converted into ferrous form from ferric state before drug administration and in this line, the use of these two plant extracts during the preparation of ABK formulation are more useful.



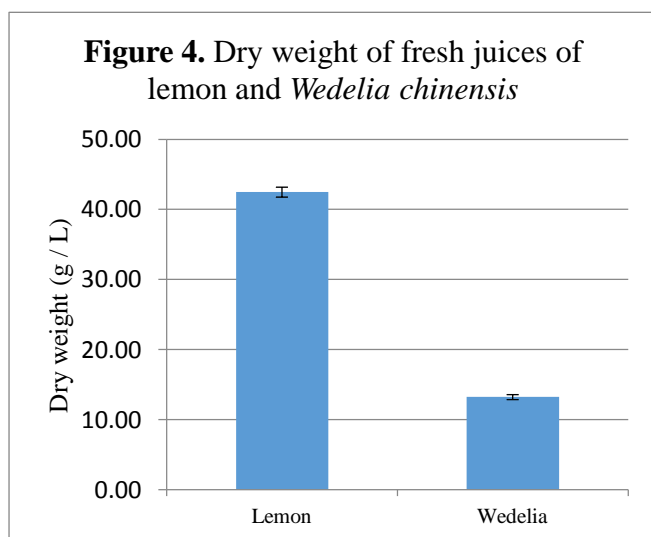
Antioxidant activity

Aqueous extract of ABK formulation exhibited moderate antioxidant activity (42.68%) at a concentration of 100 mg/ml (Figure 2). The IC-50 value of 119 mg/ml indicated that the ABK formulation has moderate antioxidant power when compared to the standard gallic acid (IC-50 = 2.34 mg/ml). Since the herbal phyto-constituents from lemon and *Wedelia chinensis* juices might have been used to reduce the iron (III) into iron (II) during the preparation of ABK formulation, the final drug showed moderate antioxidant power.



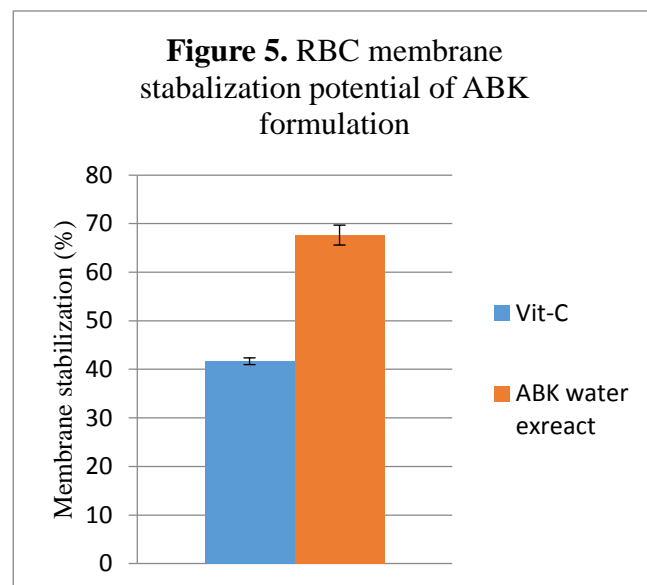
Extract yield

The extract yields of juices of lemon and *W. chinensis* were determined and the results are expressed on dry weight basis in the Figure 4. Between the two samples, lemon juice demonstrated higher level of dry weight (42.50 g / L) when compared to *W. chinensis* (13.25 g / L). Since, huge volume of lemon and *W. chinensis* juices are used during the preparation of ABK formulation, they also might add the final dry weight of the drug. But, the constituents of these juices have either been used to convert the iron from ferric to ferrous form or may be denatured due to heat treatment (sooriya pudam) during the preparation of ABK formulation.



Membrane stabilization potential

Aqueous extract of ABK formulation is found to be effective to inhibit the H₂O₂-induced oxidative damage in RBC membrane (67.65%) when compared to reference standard vitamin-C (41.67%) (Figure 5). Since the water soluble components of ABK formulation has remarkable level of RBC membrane stabilization potential, it could prevent oxidative-stress induced haemolysis and thus helpful in preventing the anaemia.



Conclusion

The plant *Eclipta alba* and *Wedelia chinensis* are commonly called as Birunkarajam therefore the drug manufacturers used either one of this plant but this research study revealed that *W.chinensis* found to be higher ferric reducing power compare to *E.alba*, hence the use of *W.chinensis* extract is more useful in the preparation of ABK. The total phenolic content as well as antioxidant activity were found to be moderate in ABK formulation, due to the fact that the herbal components were already used to convert the ferric form of iron to ferrous form during preparation. The herbal ingredients like lemon and *Wedelia chinensis* juices were incorporated in ABK preparation and their phytochemicals are either denatured during natural heating process or might be used to reduce the iron to ferrous form. However, the aqueous extract of ABK formulation showed appreciable RBC membrane stabilization effect, which could be one of the mechanisms for its anti-anaemic action.

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