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Protective capacity of *Artemisia annua* as a potent antioxidant remedy against free radical damage

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PEER REVIEW

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Comments

This is a good study in which authors examined the phyto-protective potential of *A. annua* as a natural antioxidant to combat free radicals damage in selected *in vitro* assay systems by screening four solvent leaves extracts of the plant. The extracts remarkably inhibited lipid peroxidation and erythrocyte haemolysis. Among the solvents used, 70% alcohol extracts showed better antioxidant activity than the others.
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ABSTRACT

Objective: To evaluate the antioxidant capacity of four leaf-derived solvent extracts of *Artemisia annua* (*A. annua*), a medicinal plant widely touted for its vast phyto-therapeutic potential.
Methods: *A. annua* leaves were extracted with four solvents (absolute ethanol, absolute methanol, 70% ethanol and 70% methanol), and extracts obtained studied by five complementary *in vitro* antioxidant test systems using ascorbic acid (vitamin C) and rutin as standard references.
Results: The extracts remarkably inhibited lipid peroxidation (79.81%-86.70%), and erythrocyte haemolysis (40.02%-49.91%). Their IC_{50} values for hydroxyl, nitric oxide and hydrogen peroxide radical scavenging activities ranged from 2.39-3.81 mg/mL (superior to the standards), 107.24-144.49 μg/mL and 28.53-53.20 μg/mL, respectively. 70% alcohol extracts generally showed better antioxidant activity than absolute alcohol extracts.
Conclusions: The results indicate that *A. annua* leaf extracts have potent antioxidant activities that would have beneficial effect on human health, and aqueous organic solvents are superior to the absolute counterparts in yielding extracts with better antioxidant potential.

KEYWORDS
Antioxidants, *Artemisia annua*, Free radicals, Phyto-protection

1. Introduction

Many of the diseases afflicting people today are attributable to oxidative stress that results from an imbalance between formation and neutralization of free radicals[1]. Free radicals, which are produced both endogenously and exogenously in living cells, initiate oxidative stress when they seek stability through electron pairing with biological macromolecules such as lipids, proteins, DNA and cofactors of many enzymes in healthy human cells. These activities cause lipid peroxidation, protein and DNA damage which contribute to numerous pathological disturbances including cancer, atherosclerosis, cardiovascular diseases, ageing and inflammatory diseases[2]. Much as all human cells protect themselves against free radical damage by

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antioxidant enzymes like superoxide dismutase, and catalase or compounds such as ascorbic acid, tocopherol and glutathione, in some cases, various pathological processes disrupt these protective mechanisms and natural antioxidant supplements from plants, which are presumed safer against synthetic antioxidants, are vital to combat oxidative damage.

Plants are a valuable source of natural antioxidants and constitute an important source of active natural products, many of which are used as additives in functional foods or bioactive components in pharmaceutical preparations. Various phytochemical constituents of plants, especially vitamins and polyphenols such as flavonoids, phenolic acids, phenyl propanoids, tannins, *etc.*, are known to be responsible for the free radical scavenging antioxidant activities of plants[3]. The need to optimize plant factories for sourcing natural antioxidants from the diverse medicinal and culinary plants that nature endowed us with has been strongly emphasized[4]. In Nigeria, for example, many indigenous plants have been screened for antioxidant potentials, and interestingly, even some neglected and underutilized species have shown promise as sources for cheap natural antioxidants[5,6].

Artemisia annua L. (*A. annua*), commonly called annual wormwood, and belonging to the family Asteraceae, is a medicinal plant native to temperate Asia but has presently naturalized throughout the world. It has been recently introduced for commercial cultivation in Nigeria[7]. The plant has been scientifically revealed to contain a rich portfolio of bioactive compounds including polyphenols and exhibits a wide range of pharmacological properties including antimalarial, antimicrobial, antiparasitic, and antiviral activities[8]. Artemisinin, one of the most important of the several compounds produced by the plant, is highly potent and efficacious against multidrug resistant strains of malarial parasites and has also displayed unique pharmacological activities against a wide range of parasitic organisms, a number of viruses and tumour[9]. Quite recently, *A. annua* tea infusion has been scientifically proven to show a remarkable anti-HIV activity *in vitro*[10].

A. annua tissues (such as the leaves, inflorescence, and stems) have shown great promise as good reservoirs of nutrients and antioxidants, with very minimal anti-nutritive factors which might favour their use as potential herbal tonic by humans or an important supplementary feed additive for livestock production systems[11]. This finding, coupled with the remarkably high amounts of phenols and flavonoids contained in the plant, is a significant pointer to its potential antioxidant activity. This is due to the chemical nature of these phytochemicals (phenols and flavonoids) which makes them strong reducing agents.

In an effort to further examine the phyto-protective potential of *A. annua* as a natural antioxidant in the global effort to combat free radicals damage and also justify the wide adaptability of the crop in different parts of the world, the current study was aimed at screening four solvent extracts of the plant for their antioxidant activities in selected *in vitro* assay systems.

2. Materials and methods

2.1. Plant material and extraction

Leaves of *A. annua* were obtained courtesy of Molecular Bio/Sciences Limited, 124 MCC Road, Calabar, Cross River State, Nigeria. Authentication of identity of the plant was done by Mr. Frank Adepoju, a plant taxonomist in the Department of Botany, University of Calabar and a specimen voucher deposited in the herbarium. The leaves were air-dried at room temperature and finely milled. Twenty grams of the milled sample was then accurately weighed and subjected to Soxhlet extraction with an equal volume (300 mL) of four different solvents including absolute ethanol, ethanol:water (70:30 v/v), absolute methanol, methanol:water (70:30 v/v), respectively. The resulting extracts were concentrated under vacuum in a rotary evaporator and kept in a refrigerator until needed for use. The extract yield obtained with the different solvents were 14.33%, 18.32%, 17.80% and 21.09% of starting dry plant material for absolute ethanol, 70% ethanol, absolute methanol and 70% methanol solvents respectively.

2.2. Chemicals

Folin and Ciocalteau's phenol reagent and trichloroacetic acid were purchased from Qualikems Fine Chemical Pvt. Ltd., New Delhi, India; gallic acid monohydrate from Kem Light Laboratories Pvt. Ltd., Mumbai, India while rutin was purchased from Aldrich Company Inc., USA. Solvents and other chemicals used for this study were of the highest analytical grade, while water was glass distilled.

2.3. Preparation of A. annua extracts and standard reference stock solutions

A small quantity (0.3 g) of each concentrated extract and standard references (ascorbic acid and rutin) was accurately weighed and dissolved in 100 mL of distilled water to make a sample stock solution of 3 000 μg/mL. Working concentrations of each sample were subsequently prepared as desired by appropriate dilutions.

2.4. Determination of antioxidant activity in linoleic acid system

The antioxidant activity of *A. annua* extracts was determined in this assay by measuring the extent of oxidation of linoleic acid using the ferric thiocyanate method[12]. Rutin was used as positive control.

2.5. Inhibition of hydrogen peroxide-induced erythrocytes haemolysis

Antioxidant activity of *A. annua* extracts was determined

in this assay as percentage inhibition of erythrocytes haemolysis with hydrogen peroxide as a free radical initiator[13].

2.6. Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activity of *A. annua* extracts was determined in this assay by the Fenton reaction using the ortho-phenanthroline method[14].

2.7. Nitric oxide scavenging activity

The nitric oxide scavenging ability of *A. annua* extracts was determined using Griess reaction method[15].

2.8. Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging activity of *A. annua* extracts was determined using the UV spectrophotometric method[16].

2.9. Statistical analysis

The experiment was laid out in a factorial arrangement and IC_{50} values where calculated, were obtained from a linear curve of percentage of scavenging activity versus sample concentration. Data were analyzed using analysis of variance (ANOVA). All measurements were replicated three times and results expressed as means±SE. Significant means were separated using the least significant difference analysis.

3. Results

3.1. Inhibition of linoleic acid (lipid) peroxidation

The inhibition (%) of linoleic acid peroxidation by the extracts of *A. annua* leaves, ascorbic acid and rutin, after 360 h incubation is shown in Table 1. *A. annua* extracts showed good lipid peroxidation inhibition (ranging from 79.81%-86.70%) which did not differ significantly ($P>0.05$) amongst the various treatments. The lipid peroxidation activities were also comparable ($P>0.05$) to that of a standard reference, rutin (83.33%).

Table 1
Inhibition of linoleic acid peroxidation by *A. annua* extracts, ascorbic acid and rutin after 360 h of incubation.

Extract/reference	Inhibition (%)
Absolute ethanol	79.810±2.221
Ethanol:water	84.300±1.475
Absolute methanol	80.110±3.661
Methanol:water	86.750±2.791
Rutin (reference)	83.330±3.202

Values are presented as mean±SE.

From Figure 1, it is evident that lipid peroxidation inhibition activities of all the test samples initially dropped from the 24th to 48th hour of incubation, before maintaining a gradual rise up until the end of the incubation period (360th hour).

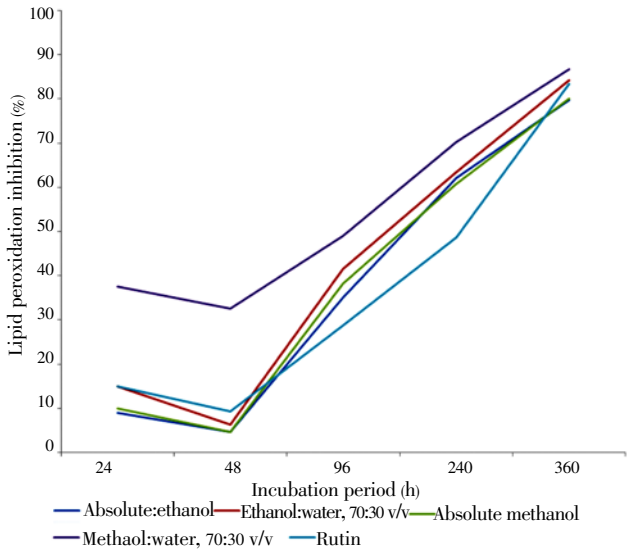

Figure 1. Time course plots for lipid peroxidation inhibition activities of *A. annua* extracts and reference compound (rutin) at various incubation periods.

3.2. Inhibition of erythrocytes haemolysis induced by hydrogen peroxide

The inhibition (%) of hydrogen peroxide-induced erythrocyte haemolysis by *A. annua* extracts and reference compounds is shown in Table 2. This ranged from 40.00% to 71.21%. Among the various treatments, the methanol:water extract showed the highest inhibition (49.91%) of erythrocyte haemolysis, though this did not differ significantly ($P>0.05$) from that of ethanol:water (46.54%). Absolute methanol and ethanol extracts showed significantly lower ($P<0.05$) inhibition of erythrocyte haemolysis (43.18% and 40.00%), respectively. These values, however, were significantly lower than those of ascorbic acid and rutin (71.21% and 63.55%, respectively) used as standard references. Generally, the extracts and reference compounds showed a dose-dependent increase in inhibition percentage from 50-250 μg/mL (Figure 2).

Table 2
Inhibition of erythrocytes haemolysis by *A. annua* extracts and reference compounds.

Extract/standard	Inhibition (%) of erythrocytes haemolysis
Absolute ethanol	40.00±14.03
Ethanol:water	46.54±9.43
Absolute methanol	43.18±12.05
Methanol:water	49.91±9.44
Ascorbic acid (reference)	71.21±9.90
Rutin (reference)	63.55±8.02
LSD	3.082

Values are presented as mean±SE. LSD: least significant difference.

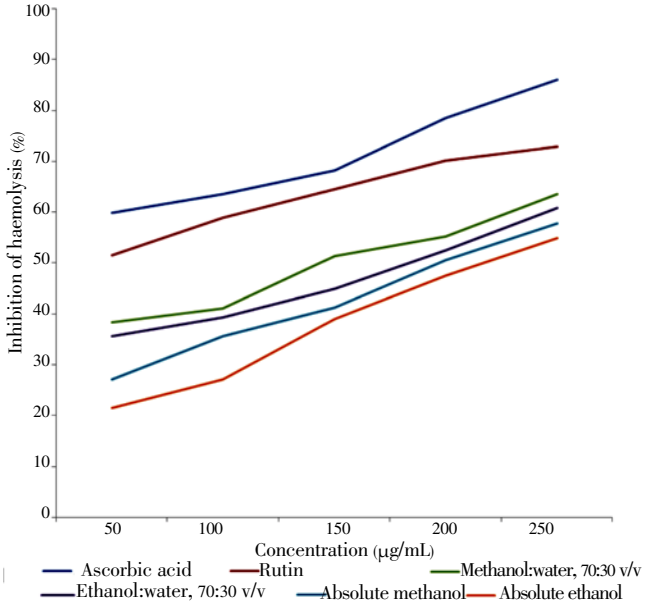

Figure 2. Percentage inhibition of erythrocytes haemolysis by *A. annua* extracts and references at different tested concentrations.

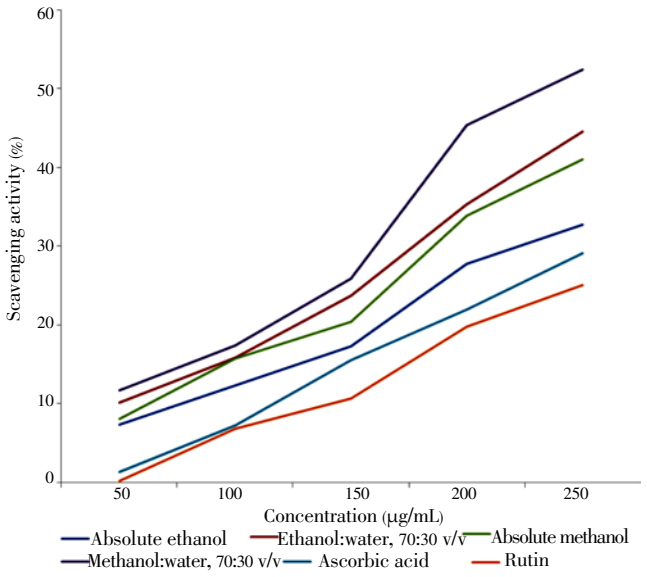

Figure 3. Hydroxyl radical scavenging activity of *A. annua* extracts and reference compounds at different concentrations tested.

3.3. Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activities (expressed as IC_{50}) of *A. annua* extracts, ascorbic acid and rutin as shown in Table 3 ranged from 2.39–4.53 mg/mL. Amongst the extracts, methanol:water showed the highest scavenging activity with the lowest IC_{50} value (IC_{50}=2.39 mg/mL), which did not differ significantly (P>0.05) from those of ethanol:water and absolute methanol extracts (IC_{50} values are 2.76 mg/mL and 3.02 mg/mL, respectively). Absolute ethanol extracts had the least scavenging activity (IC_{50}=3.81 mg/mL), which was not significantly different (P>0.05) from the scavenging activities of ascorbic acid and rutin (IC_{50} values are 3.98 mg/mL and 4.53 mg/mL, respectively). Generally, the hydroxyl radical scavenging activities of the extracts which were superior to those of the reference compounds (ascorbic acid and rutin) followed a dose dependent pattern (increased with increasing doses from 0.5–2.5 mg/mL), as evident from Figure 3.

Table 3

Concentration of *A. annua* extracts and reference samples necessary to scavenge 50% of hydroxyl, nitric oxide and hydrogen peroxide radicals.

Extract/reference	OH	NO	H_2O_2
	IC_{50} (mg/mL)	IC_{50} (μg/mL)	IC_{50} (μg/mL)
Absolute ethanol	3.810^{a}±0.147	138.910^{d}±1.628	53.200^{e}±1.227
Ethanol:water	2.760^{b}±0.156	122.230^{c}±1.814	34.290^{d}±1.722
Absolute methanol	3.020^{b}±0.059	144.490^{d}±2.118	53.040^{e}±1.040
Methanol:water	2.390^{b}±0.083	107.240^{b}±1.450	28.530^{c}±1.255
Ascorbic acid	3.980^{a}±0.200	–	9.600^{a}±0.283
Rutin	4.530^{a}±0.314	22.050^{a}±0.926	17.230^{b}±0.811
LSD	0.780	5.156	3.529

Values are presented as mean±SE. Means with same superscripts in each column do not differ significantly (P>0.05) from one another. IC_{50}: scavenge 50%, OH: hydroxyl, NO: nitric oxide, H_2O_2: hydrogen peroxide, LSD: least significant difference.

3.4. Nitric oxide scavenging activity

The nitric oxide scavenging activities of the *A. annua* extracts and rutin, expressed as IC_{50} values are presented in Table 3 and ranged from 22.05–144.49 μg/mL. Methanol:water showed significantly higher (P<0.05) nitric oxide scavenging ability (IC_{50}=107.24 μg/mL) than ethanol: water (IC_{50}=122.23 μg/mL). Absolute methanol and absolute ethanol extracts showed significantly lower nitric oxide scavenging activities than the aqueous organic solvent extracts but do not differ significantly (P>0.05) among themselves (IC_{50}=144.49 μg/mL and 138.91 μg/mL, respectively). However, the nitric oxide scavenging activities of the *A. annua* extracts were not comparable (P<0.05) with that of the standard reference compound, rutin (IC_{50}=22.05 μg/mL). Generally, all the test samples showed nitric oxide scavenging activities in a dose dependent manner as evident from Figure 4.

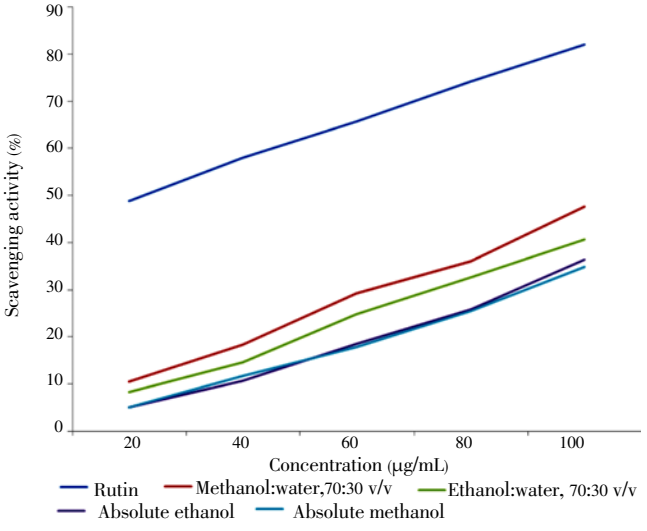

Figure 4. Nitric oxide scavenging activity of *A. annua* extracts and reference compounds at different concentrations tested.

3.5. Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging activities (IC_{50}) of *A. annua* extracts and reference compounds are shown in Table 3 and ranged from 9.60-53.20 μg/mL. Amongst the extracts, methanol:water showed the highest scavenging activity (IC_{50}=28.53 μg/mL), followed by ethanol:water (IC_{50}=34.29 μg/mL). Absolute methanol and absolute ethanol extracts showed significantly lower (P<0.05) scavenging activity than the aqueous organic solvent extracts though this did not differ significantly (P>0.05) from each other (IC_{50} values are 53.04 μg/mL and 53.20 μg/mL, respectively). However, ascorbic acid and rutin showed significantly higher (P<0.05) scavenging activities (IC_{50} values are 9.60 μg/mL and 17.23 μg/mL, respectively) than all the extracts. Again, generally, hydrogen peroxide scavenging activities of all the test samples maintained a dose dependent increase from 20-100 μg/mL (Figure 5).

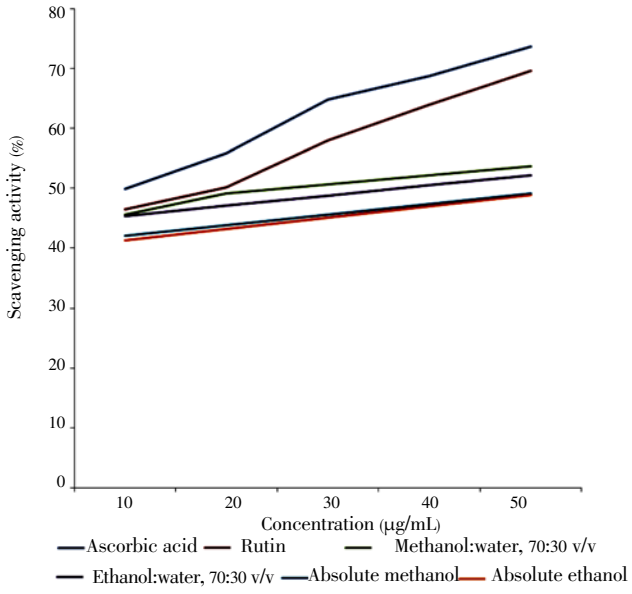

Figure 5. Hydrogen peroxide scavenging activity of *A. annua* extracts and reference compounds at the different concentrations tested.

4. Discussion

A. annua ranks high among medicinal plants having the capacity to offer phyto-protective activity against numerous diseases of humans and animals. This potential is conferred on it undoubtedly by its rich portfolio of medicinally important bioactive compounds including several sesquiterpenoids and antioxidants. The concerted global search for naturally-occurring antioxidants to replace the widely criticized synthetic counterparts in combating free radical damage has again drawn our attention to this seemingly innocuous yet highly versatile plant whose high amounts of phenols and flavonoids may lend credence to its potent antioxidant capacity.

The ferric thiocyanate method is commonly used to evaluate the antioxidant activity in food and biological systems. It measures the extent of suppression of lipid peroxidation (peroxide compounds formed during lipid oxidation) by antioxidant compounds in test samples. *A. annua* leaf extracts showed remarkably good linoleic acid peroxidation inhibition activities (79.81%-86.75%) after 360 h of incubation. These values compared very well with that of rutin (83.33%) used as a reference, and of *Moringa oleifera* and *Aloe barbadensis* leaves (73.3%-86.2% and 68.2%-82.6%), respectively[17], thus serving as a possible evidence to the potency of the plant in conferring protection to human cells against free-radical induced peroxidation of lipids. Lipids such as free and ester forms of polyunsaturated fatty acids and cholesterol are vulnerable targets of free radicals[18]. Lipid peroxidation can be stimulated by transition metals such as iron through the Fenton reaction, and can be accelerated when the iron decomposes lipid peroxides into peroxyl and alkoxyl radicals that can perpetuate the chain reaction. Peroxidation of lipids has been reported to induce disturbance and alteration of biological membranes, and many studies have shown the association between free radical mediated lipid peroxidation products and the progress of diseases[19].

The ability of *A. annua* leaf extracts to inhibit haemolysis of erythrocytes induced by hydrogen peroxide is another pointer to its possible protective effect against red blood cell damage by appreciably down-regulating oxidative damages done to these cells due to the activities of free radicals, particularly reactive oxygen species. This confirms, invariably, the reducing potential of the plant material. Interestingly, it has been observed that a good antioxidant must act as a good reducing agent. Again, the better inhibition activity of the aqueous solvent extracts against the absolute counterparts may be explained as consequent on the more efficient extraction of antioxidant compounds by the more polarized aqueous solvent mixtures. Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide crosses cell membranes and reacts with ferric and copper ions, which shows toxic effects. Erythrocytes (red blood cells) have been used extensively used to study oxidative damages. The red blood cell is unique among cells in that it combines very large concentrations of both iron (haemoglobin) and oxygen. This potentially dangerous combination of oxygen and iron within the red blood cells makes it powerful promoters of oxidative processes. Red blood cells are extremely susceptible to oxidative damages to polyunsaturated fatty acids of their membranes.

A. annua leaf extracts scavenged hydroxyl radicals at the tested concentrations in a dose dependent manner and interestingly, superior to the reference compounds used. The hydroxyl radical is a highly reactive oxidizing species

that can be generated by some metal catalyzed oxidation reactions, for example, the interaction of hydrogen peroxide (H_2O_2) and superoxide radicals in the presence of certain transition metal ions[20]. The hydroxyl radicals produced through such metal catalyzed oxidation reactions are the major active oxygen species that cause peroxidation of membrane lipids, attack all proteins and any biomolecule they touch, causing enormous biological damage. In *in vitro* studies, hydroxyl radicals are usually generated via the Fenton reaction ($Fe^{2+}+H_2O_2 \longrightarrow Fe^{3+}+\cdot OH+OH^-$) in which iron (II) sulphate (which generates ferrous ions, Fe^{2+}) is mixed with hydrogen peroxide and 1, 10 phenanthroline. The 1, 10 phenanthroline is used since phenanthroline-Fe^{2+} is a commonly used indicator of redox reaction. The H_2O_2/Fe^{2+} system produces hydroxyl radicals through the Fenton reaction and phenanthroline-Fe^{2+} complex oxidizes to Fe^{3+}. The hydroxyl radical produced can then be determined due to a change in absorbance. The ability of *A. annua* extracts to scavenge hydroxyl radicals better than ascorbic acid and rutin used as references in this study may be an interestingly clear signal that they may also prevent bio membranes and bio molecules from being attacked by free radicals.

Nitric oxide is a small gas molecule made by many different cells, which functions in hormone regulation and control of blood pressure[21]. Sustained levels of production of this gas make it a radical that is directly toxic to tissues and contribute to the vascular collapse associated with septic shock, whereas chronic expression (excess generation) of nitric oxide radical in the human body is associated with various carcinomas and inflammatory conditions including juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis[22]. The results of this study is indicative that *A. annua* extracts, though not comparable with standard rutin, were able to inhibit nitrite formation by directly competing with oxygen in the reaction with nitric oxide. This may therefore be a pointer to the fact that the plant extracts may have the property to counteract the effect of nitric oxide formation and consequently, could potentially be beneficial against excessive nitric oxide generation in the human body which has attendant health consequences.

Scavenging of hydrogen peroxide by the plant extracts has been partly attributed to their phenolics, which can donate electrons to H_2O_2, thus neutralizing it to water. The *A. annua* extracts used in the current study were capable of scavenging hydrogen peroxide in a concentration-dependent manner. Hydrogen peroxide is mildly toxic but can potentially cause cytotoxicity through production of highly reactive hydroxyl radicals when it reacts with Fe^{2+} and possibly Cu^{2+} ions on crossing cell membranes. The ability of the tested extracts to scavenge H_2O_2 makes them potentially useful in biological and food systems.

On the basis of the results presented in this study, it can be concluded that leaf extracts of *A. annua* exhibited very good but varying levels of antioxidant activities in all the models studied. This provides evidence to the potency of the plant as a natural source of antioxidants that can be exploited for use as industrial raw materials for the production of functional nutraceuticals. It also brings to light the efficiency of aqueous organic solvent extraction for recovery of maximum antioxidant compounds and obtaining potent antioxidant activities in *A. annua* leaves. However, further research is needed to test the plant material *in vivo* and identify individual components forming antioxidant system in the plant for their development as products for food and pharmaceutical industries.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Free radicals pose serious health challenges globally by causing oxidative damage to healthy human cells. Antioxidants are molecules that can safely interact with these radicals and down-regulate their hazardous effect. From the point of view of safety, natural antioxidants of plant origin are advocated for use in combating the myriad of pathological disturbances induced by free radicals.

Research frontiers

In vitro screening of plant extracts for natural antioxidant potential is considered a vital preliminary step towards unraveling good candidates for possible pharmaceutical and nutraceutical exploitation. The present study highlights the natural antioxidant potentials of different solvent extracts of *A. annua*-an antimalarial plant recently introduced into Africa.

Related reports

A. annua tissues (such as the leaves, inflorescence, and stems) have shown great promise as good reservoirs of nutrients and antioxidants, with very minimal anti-nutritive factors which might favour their use as potential herbal tonic by humans or an important supplementary

feed additive for livestock production systems.

Innovations and breakthroughs

A. annua is an important medicinal plant widely used for the treatment of malaria and other diseases especially in Africa. In the present study, authors have demonstrated the use of *A. annua* leaf extract to remove free radicals intermediates, which can cause damage and death to cells.

Applications

A. annua is at the centre of interest in Africa due to its efficacy in the treatment of malaria. The results of this study suggest that the plant is quite adapted to African climate and can be of additional antioxidant benefits (especially when extracted with alcohol:water mixture) especially for people who drink the extract as tea. Its inclusion into animal diet would likely boost the antioxidant principles.

Peer review

This is a good study in which authors examined the phyto-protective potential of *A. annua* as a natural antioxidant to combat free radicals damage in selected *in vitro* assay systems by screening four solvent leaves extracts of the plant. The extracts remarkably inhibited lipid peroxidation and erythrocyte haemolysis. Among the solvents used, 70% alcohol extracts showed better antioxidant activity than the others.

References

[1] Abheri DS, Gosh AK, Mallick AR. Free radicals and their role in different clinical conditions: an overview. *Int J Pharm Sci Res* 2010; **3**: 185-192.

[2] Tripathy S, Pradhan D, Anjana M. Anti-inflammatory and antiarthritic potential of *Ammania baccifera* Linn. *Int J Pharm Bio Sci* 2010; **1**(3): 1-7.

[3] Aiyegoro OA, Okoh AI. Preliminary phytochemical screening and *in vitro* antioxidant activity of the aqueous extract of *Helichrysum longifolium* DC. *BMC Complement Altern Med* 2010; **10**: 21-32.

[4] Ikpeme EV, Udensi OU, Ekerette EE, Chukwurah PN. Optimization of plant factory for sourcing natural antioxidants-a paradigm shift. *Int J Adv Res* 2013; **1**(4): 7-15.

[5] Uyoh EA, Chukwurah PN, David IA, Bassey AC. Evaluation of antioxidant capacity of two *Ocimum* species consumed locally as spices in Nigeria as a justification for increased domestication. *Am J Plant Sci* 2013; **4**: 222-230.

[6] Uyoh EA, Chukwurah PN, Akarika RC, Antia VA. Potentials of two Nigerian spices-*Piper nigrum* and *Monodora myristica* as sources for cheap natural antioxidants. *Am J Plant Sci* 2013; **4**: 1105-1115.

[7] Brisibe EA, Udensi O, Chukwurah PN, Magalhães PM, Figueira GM, Ferreira JF. Adaptation and agronomic performance of *Artemisia annua* L. under lowland humid tropical conditions. *Ind Crops Prod* 2012; **39**: 190-197.

[8] Efferth T, Romero MR, Wolf DG, Stamminger T, Marin JJ, Marschall M. The antiviral activities of artemisinin and artesunate. *Clin Infect Dis* 2008; **47**: 804-811.

[9] Efferth T, Dunstan H, Sauerberry A, Miyachi H, Chitambar CR. Antimalarial artesunate is also active against cancer. *Int J Oncol* 2001; **18**: 767-773.

[10] Lubbe A, Seibert I, Klimkait T, van der Kooy F. Ethnopharmacology in overdrive: the remarkable anti-HIV activity of *Artemisia annua*. *J Ethnopharmacol* 2012; **141**(3): 854-859.

[11] Brisibe EA, Umoren UE, Brisibe F, Magalhaes PM, Ferreira JF, Luthria D, et al. Nutritional characterization and antioxidant capacity of different tissues of *Artemisia annua* L. *Food Chem* 2009; **115**: 1240-1246.

[12] Iqbal S, Bhanger MI, Anwar A. Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan. *Food Chem* 2005; **93**: 265-272.

[13] Fijesh PV, Jaimsha-Rani VK, Vineesh VR, Padikkala J. Antioxidant activities of *Hibiscus furcatus* Roxb. ex DC. extracts. *Res J Biol Sci* 2010; **5**(3): 269-274.

[14] Nagulendran KR, Velavan S, Mahesh R, Begum VH. *In vitro* antioxidant activity and total polyphenolic content of *Cyperus rotundus* rhizomes. *J Chem* 2007; **4**: 440-449.

[15] Rajasekhar MD, Ramesh BK, Vinay K, Sampath MR, Sameena SK, Apparao C. Antihyperglycemic and antioxidant activities of active fraction from the aqueous extract of *Momordice cymbalaria* fruits in streptozotocin induced diabetic rats. *Pharmacogn Res* 2009; **1**: 352-358.

[16] Ebrahimzadeh MA, Ehsanifar S, Eslami B. *Sambucus ebulus* elburensis fruits: a good source for antioxidants. *Pharmacogn Mag* 2009; **4**(19): 213-218.

[17] Sultana B, Anwar F, Ashraf M. Effects of extraction solvent/technique on the antioxidant activity of some medicinal plant extracts. *Molecules* 2009; **14**: 2167-2180.

[18] Niki E. Assessment of antioxidant capacity *in vitro* and *in vivo*. *Free Radic Biol Med* 2010; **49**: 503-515.

[19] Lee CY, Seet RC, Huang SH, Long LH, Halliwell B. Different patterns of oxidized lipid products in plasma and urine of dengue fever, stroke, and Parkinson's disease patients: cautions in the use of biomarkers of oxidative stress. *Antioxid Redox Signal* 2009; **11**: 407-420.

[20] Okoko T. Chromatographic characterization, *in vitro* antioxidant and free radical scavenging activities of *Garcinia kola* seeds. *Afr J Biotechnol* 2009; **8**(24): 7133-7137.

[21] Guzik TJ, West NE, Pillai R, Taggart DP, Channon KM. Nitric oxide modulates superoxide release and peroxynitrite formation in human blood vessels. *Hypertension* 2002; **39**(6): 1088-1094.

[22] Ebrahimzadeh MA, Nabavi SF, Nabavi SM, Eslami B, Asgarirad H. *In vitro* antioxidant and free radical scavenging activity of *Leonurus cardiaca* subsp. Persicus, *Grammosciadium platycarpum* and *Onosma demawendicum*. *Afr J Biotechnol* 2010; **9**(51): 8865-8871.