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Research Article

Antioxidant Potential of Few Medicinal Plants: A New Hope in Pharmaceuticals

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Abstract: Traditional medicine exploiting the natural resources has been the main stay of several ethical tribes. The ability to scavenge for free radicals by phenolic compounds is due to possession of a phenolic hydroxyl group which is associated with therapeutic action against free radical mediated diseases attracting much research interest. The plant extracts show considerable amount of antioxidant activity.In the present investigation, the aquous acidic extract of O. sanctum and A. mexicana revealed the high antioxidant activity and it can be correlated with the high amount of phenolic acids detected in the aquous acidic extracts. The alcoholic extract of A. tribuloides also revealed high inhibition percentage.

Keywords: corrosion, inhibitors, vernonia amygdalina, adsorption.

INTRODUCTION

Natural products play a dominant role in pharmaceutical industry and systemic investigation of natural resources for the discovery of new drug molecules has been a primary objective for bio prospection programs[1]. Traditional medicine or folk medicine practice is based on the use of living material (animals, plants, insects or microbes). There are about 127 natural products natural product derived compounds currently undergoing clinical trials. Currently, microbial resistance to antibiotics has become a global concern as it spans all known classes of natural and synthetic compound fueling the need of new principles of therapy[2-5] . In addition to protection against biotic stresses, secondary metabolite is also generated in response to abiotic stresses. Several reactive oxygen(ROS) species are continuously produced in plants as byproducts of aerobic metabolism, abiotic stress conditions and signaling molecules

in pathogen defense, programmed cell death etc[6] . Secondary metabolites, especially phenylpropanoid acts as antioxidant to overcome the harmful effect of reactive oxygen species in addition to enzymes such as catalase, guaicol peroxidase, amylase and phosphatase. Incidentally, it is increasingly being realized that many of the modern human diseases are due to 'oxidative stresses initiated by free radicals [7,8]. During normal metabolic functions, highly reactive compounds called free radicals are created in the body. However, free radicals may also be introduced from the environment. These compounds are inherently unstable since they have an odd number of electrons. To make up for their shortage in electrons, these free radicals will react with certain chemicals in the body, and in so doing, they interfere with the cell's ability to function normally. As such development of ethnomedicines from plants with strong antioxidants properties has received much attention.

A biological antioxidant has been defined as "any substance that, when present at low concentrations compared to those of anoxidizable substrate, significantly delays or prevents oxidationof that substrate" [9]. Oxidative damage is the cause of many diseases includingcancer [10], liver disease[11] , Alzheimer's disease [12], aging[13] , arthritis [14], inflammation[15] , diabetes [16],[17], Parkinson'sdisease [18],[19], , atherosclerosis [20]., and AIDS [21]. As such,antioxidants that preventoxidative damage are a potent source of treatment.

MATERIALS AND METHODS

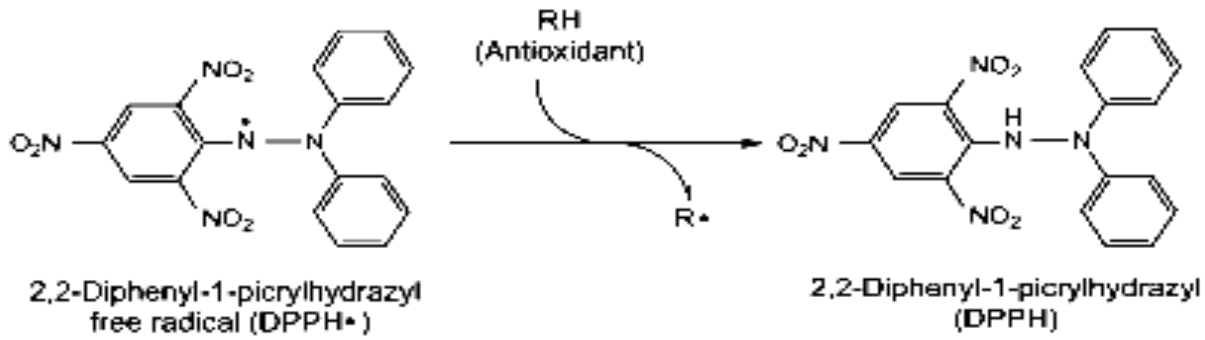
The plant used for study was collected. The species information was correlated with USDA data base (USDA, ARS, National Genetic Resource programme, 2009). Unless otherwise mentioned, all experiments were performed with fresh leaves. The plant used for the above study were *Ocimum sanctum*, *Calotropis procera*, *Astragalus tribuloids* and *Argemone Mexicana*

Antioxidant assay:

DPPH radical scavenging activity: The method of Liyana-Pathiranan and Shahidi[22] was used for the determination of scavenging activity of DPPH (1, 1 dihydroxy 2-picrylhydrazyl, Sigma Aldrich) free radical in the extract solution. A solution of 0.135mM DPPH in methanol was prepared and 1.0 ml of solution was mixed with 30 µl of extract prepared in methanol containing 0.025-0.5 mg of the plant extract and standard separately (BHT). The reaction mixture was vortexed thoroughly and left in a dark for 30 min. The absorbance of the mixture was measured Spectrophotometrically at 517 nm.

Hydroxyl radical scavenging: This was assayed as described by [23] with a slight modification. This assay is based on quantification of degradation product of 2-deoxyriobose by condensation with TBA. Hydroxyl radical was generated by the Fe3+-ascorbate- DTA-H2O2 system (Fenton reaction). The reaction mixture contained, in a final volume of 1ml, 2-deoxy-2-ribose (2.8mM); KH_2PO_4 - KOH buffer (20mM, pH7.4); $FeCl_3$ (100mM); EDTA (100µM); H_2O_2 (1.0mM); ascorbic acid(100µM) and various concentrations (0-200µg/ml) of the of the test sample. After incubation for 1 hr.at 37^0C, 0.5ml of the reaction mixture was added to 1ml of 2.8% TCA, then 1ml of 1% TBA was added and the reaction mixture was incubated at 90^0C for 15min to develop the color. After cooling, the absorbance was measured at 532nm against an appropriate blank solution. All tests were performed three times. Mannitol, a classical OH scavenger, was used as positive control. Percentage inhibition was evaluated by comparing the test and blank solution.

Reducing power: The Fe^{3+}-reducing power of the extract was determined by the method of oyaizu [24] with a slight modification.aliquots (2.5ml) of the sample extracts in phosphate buffer (0.2m phosphate buffer, ph 6.6) was added to 2.5ml of potassium ferricynide (10mg/ml) and the reaction mixture was incubated at 500c for 20min. Tca (2.5ml of 100mg/ml solution) was then added to the reaction mixture, vortexed and centrifuged at 1000 rpm for 10min. The resultant supernatant (2.5ml) was mixed with equal volume of distilled water and 0.5ml of ferric chloride was added (1mg/ml solution). Absorbance was measured spectrophotometrically at 700nm against ascorbic acid standard and higher absorbance of sample indicated greater reducing power.



RESULTS AND DISCUSSIONS

The results of various assays for the antioxidant potential for the medicinal plants are as follows:

DPPH radical scavenging activity of Phenolic extracts (alchoholic, aquous acidic and alkaline) expressed as percentage inhibition.

Plants	Inhibition (%)		
	Alcoholic	Aq. acidic	Alkaline
Ocimum sanctum	41.70±2.91^{bc}	48.46±2.93^{a}	30.02±1.57^{d}
Calotropis procera	11.94±4.00^{f}	40.17±2.16^{c}	27.43±1.40^{d}
Astragalous tribuloides	49.72±5.49^{a}	40.02±1.77^{c}	47.08±5.76^{ab}
Argemone mexicana	37.47±2.12^{c}	49.62±1.72^{a}	20.24±2.74^{e}

*** Values followed by the same alphabet in the table are statistically not significantly different at *p*=0.05 following ANOVA and DMRT**

Hydroxyl radical scavenging activity of the plant extracts (% inhibition in mannitol 57%)

Plants	Inhibition (%)		
	Alcoholic	Aq. Acidic	Alkaline
Ocimum sanctum	36.33±1.53^{ab}	14.33±2.52^{h}	19.67±5.03^{gh}
Calotropis procera	17.33±3.51^{gh}	15.33±1.53^{h}	25.67±3.51^{de}
Astragalous tribuloides	21.67±3.06^{efg}	31.67±1.53^{bc}	40.67±4.51^{a}
Argemone mexicana	30.67±3.06^{cd}	17.67±1.53^{gh}	23.67±4.04^{ef}

*** Values followed by the same alphabet in the table are statistically not significantly different at *p*=0.05 following ANOVA and DMRT**

Reducing power of plant extracts in terms of ascorbic acid equivalents.

Plants	Reducing power (µg/ ml ascorbic acid equivalent)		
	Alcoholic	Aq. Acidic	Alkaline
Ocimum basilicum	1.38±0.06[c]	1.03±0.06[d]	0.08±0.02[f]
Calotropi sprocera	0.21±0.04f	0.95±0.04[d]	0.10±0.01[f]
Astragalous tribuloides	1.34±0.22[c]	2.42±0.07[f]	2.96±0.25[a]
Argemone mexicana	0.60±0.04[e]	1.09±0.17[d]	0.25±0.04[f]

*** Values followed by the same alphabet in a column are statistically not significantly different at *p*=0.05 following ANOVA and DMRT**

Phenolic compounds are vital plant secondary metabolites that can help plants tide over oxidative stress working as antioxidants[25,26] . Free radical generation is directly related with oxidation in food and biological systems and a number of methods are available for the determination of free radical scavenging, but they are case specific [27,28] .

In the DPPH assay, butylated hydroxytoluene (BHT) was used as a standard whereas for hydroxyl radical scavenging assay, mannitol was used. Reducing power was expressed as ascorbic acid equivalents.The findings show the importance of using more than one antioxidant assay method to validate the results obtained. The DPPH assay is quite popular as it is one of the few stable and commercially available organic nitrogen radicals[29].

The plant extracts show considerable amount of antioxidant activity. The aquouss acidic extracts of *O. sanctum* and *A. mexicana* show the high antioxidant activity in DPPH assays and it can be correlated with the high amount of phenolic acids detected in the aq. acidic extracts. The alcoholic extract of *A. tribuloides* also shows high inhibition percentage.

In the hydroxyl radical scavenging activity however, the alcoholic extracts of *O. sanctum* and *A. mexicana* show high inhibition. Higher inhibition percentage is also seen in the aq. acidic and alkaline extracts of *A. tribuloides*. The aq. acidic extracts, except for *A. tribuloides* show low inhibition percentage.

As regards to reducing power, *A. tribuloides* show better results for all the three extracts whereas *C. procera* and *A. mexicana* are less potent. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activities of antioxidants have been attributed to various mechanisms such as prevention of chain initiation, decomposition of peroxides, reducing capacity and radical scavenging [30] . The reducing power of the plant extracts was comparable with the standard in the present study.

Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions. The ability to scavenge for free radicals by phenolic compounds is due to possession of a phenolic hydroxyl group which is associated with therapeutic action against free radical mediated diseases attracting much research interest [31]. Free radicals are known to contribute to numerous disorders in humans including cancer, artheroscerolosis, arthritis, ischemia, Central Nervous System (CNS) injury, gastritis, dementia, renal disorders and Acquired Immune Deficiency Syndrome (AIDS) [32,33]., . Free radicals are constantly generated due to environmental pollutants, radiation, chemicals, toxins, physical stress and the oxidation process of drugs and food. Many plant phenollics have been reputed to have antioxidant properties that are even much stronger than vitamins E and C. In addition, currently available synthetic antioxidant like butylated

hydroxyl anisole (BHA), butylatedhydroxytoluene (BHT) and gallic acid esters have been suspected to cause or prompt negative health effects and hence the need to substitute them with naturally occurring antioxidants [3134,33] .

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