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Original Article

SCREENING FOR BIOACTIVES FROM INDIAN MEDICINAL HERBS–A SIMPLISTIC APPROACH FOR ANTIOXIDANT METABOLITES

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ABSTRACT

Herbal extracts obtained from 20 Indian medicinal plants were evaluated for their cytoprotectivity on erythrocytes and antioxidant properties. Total phenol content and anti-rhizopus activity were also determined. Results indicated that, out of 20 extracts evaluated, radical scavenging capacity and anti-rhizopus activity were observed in aqueous extract of *Ocimum tenuiflorum, Leucas aspera, Terminalia arjuna, Glycyrrhiza glabra* and *Nyctanthes arbortristis* in a dose dependent manner. The total phenolic content was observed to be 1289, 3837, 372, 2831 and 1892 µg GAE/g for *O. tenuiflorum, L. aspera, T. arjuna, G. glabra* and *N. arbortristis* respectively. The antioxidant activity correlates with the phenolic content of the extracts. At 1 mg/ml the above extracts showed 98% protection on erythrocyte cell oxidation. These results demonstrate that the cytoprotectivity and antioxidant potency of these extracts could be the basis for their alleged health promoting potential. These herbs could serve as new sources of natural antioxidants or nutraceuticals with potential applications in reducing oxidative stress conditions.

Keywords: Herbal extracts, Cytoprotective, Antioxidant, Anti-rhizopus, DPPH, Oxidation.

INTRODUCTION

Many studies have established the relation between oxidative stress, cellular senescence, metabolic disorders and diseases [1]. In physiological and pathological pathways, reactive oxygen species can cause DNA mutation, protein oxidation and lipid peroxidation, contributing to the development of atherosclerosis, inflammation, neurodegenerative diseases, cataracts, cancer and aging [2]. Free radicals are generated during normal cellular metabolism and their effect is neutralized by antioxidant molecules present in the body. However, the balance between the oxidants and antioxidant molecules is disturbed by excess free radicals derived from exogenous sources like ozone, exposure to UV radiations and cigarette smoke [3]. A potent scavenger or quencher of these free radical species may serve as a possible preventive measure for free radical mediated diseases. There is growing concern over synthetic additives, such as butyl hydroxyanisole (BHA) and butyl hydroxy toluene (BHT), which have long been used to preserve food from oxidation [4,5]. Therefore, there is interest in natural products and plant extracts as possible sources of free radical scavengers in form of food additives.

Fruits and vegetables get infected by pathogen during harvesting and packing. *Rhizopus stolonifer* is reported to cause food spoilage and decay in fruits, particularly peaches, strawberries, raspberries and grapes usually after harvest [6]. The post-harvest losses derived from rhizopus rot are fastly increased by the spread of the fungus to the adjacent fruits during ripening because the pathogen is not efficiently controlled by registered fungicides and treatments. In an attempt to reduce the use of chemicals as preservatives, due to the concern about human health and environmental pollution, new approaches are being developed such as the use of medicinal and aromatic plant extracts with antimicrobial and antifungal activities. In the present investigation, the protective effect of herbal extracts on free radical induced damage on erythrocytes was assessed along with 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging ability. The total phenol content and anti-rhizopus potential was also determined.

MATERIALS AND METHODS

Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, and Butyl hydroxyanisole (BHA) were purchased from Hi Media Laboratories Pvt Ltd., Mumbai, India. Vanillin was purchased from Merck, India. All other chemicals and solvents were of analytical grade and purchased from common sources.

Plant materials and extraction

The plants were collected in the Northern and southern provinces in India. Plant materials (table 1) were authenticated at the Department of Studies in Botany, University of Mysore, Mysore. Each plant material was air dried in the dark and ground to powder thereafter. Around 5 g each of different parts of the plants was successively macerated at 4 °C for 24 h with 200 ml of water until exhaustion of the material. The aqueous extracts were separately lyophilized. Dry extracts were stored in glass vials at 4 °C until tested and analyzed.

Determination of total phenolic content

The total phenolic content of each of the herbal extracts was determined colorimetrically using the Folin-Ciocalteu method [1]. A sample aliquot of 100 µl was added to 900 µl of water, 5 ml of 0.2 N Folin-Ciocalteu reagent and 4 ml of saturated sodium carbonate solution (100 g/l). The absorbance was measured at 765 nm after incubation for 2 h at room temperature. The total phenolic content was expressed as gallic acid equivalent (GAE) in milligrams per gram sample.

DPPH radical scavenging assay

The effect of different herbal extracts on DPPH radical was estimated [1]. Herbal extracts (0-62.5 µg/ml) in 200 µl aliquot was mixed with 100 mM Tris-HCl buffer (800 µl, pH 7.4) and then added to 1 ml of 500 µM DPPH in ethanol (final concentration of 250 µM) and left to stand for 20 min at room temperature. The absorbance of the resulting solution was measured spectrophotometrically at 517

nm. The capacity to scavenge DPPH radical was calculated using the following equation: Scavenging effect (%) = $[(A_{control}-A_{sample})/A_{control}] \times 100$

Where A=Absorbance

Cytoprotective studies of herbal extracts on erythrocytes

Erythrocytes were obtained from healthy consenting donors. Heparinised blood was centrifuged at 1000g for 15 min. After removal of plasma and buffy coat, the erythrocytes were washed thrice with PBS (20 mM, pH 7.4, NaCl–0.9%) at room temperature and re-suspended in PBS four times its volume for subsequent analysis [9]. Erythrocytes were incubated with aqueous extracts (1 mg/ml) for 5 min and then hydrogen peroxide (30 mM), ferric chloride (80 µM) and ascorbic acid (50 µM) were added and incubated at 37 °C for 1 h. The reaction mixture was gently shaken while being incubated. The morphology of erythrocytes was observed in an optical microscope.

Evaluation of antifungal activity

The fungus used in the assay was *Rhizopus stolonifer* and it was acquired from Microbial Type Culture Collection (MTCC 2189). *R. stolonifer* was grown on potato-dextrose agar (PDA) medium on Petri plates. The antifungal activity was evaluated by the method of mycelial growth [10]. Plants extracts were re-suspended in sterile eppendorff with DMSO and were added to sterile PDA medium. All extracts were assayed in a final concentration of 250 ppm. Benzoic acid and BHA were used as positive controls at the same concentration. 5 mm of *R. stolonifer* mycelium (taken from a 5 d-old fungi culture) were inoculated in fresh medium containing DMSO (control), and in fresh medium containing DMSO plus plant extract. After incubation for 24 h at 25 °C in darkness, growth zones were measured and converted into the percentage of inhibition: [(Control–Treated)/Control) × 100].

Statistical analysis

All experiments were carried out in triplicates and data were shown as mean±standard deviation (SD). SPSS 10.0.5 version for windows (SPSS software Inc., USA) computer programme was used for statistical analysis. The significance of the study was assessed by one way ANOVA, followed by Post hoc comparison test. Correlations between quantitative properties were evaluated by calculating the Duncan and Dunnett's coefficient. Statistical significance value set at $p<0.05$.

Table 1: DPPH radical scavenging potential of herbal extracts

S. ID	Taxon	Family	Part	IC_{50} (µg/ml)
1	*Ocimum tenuiflorum*	Lamiaceae	Leaf	13.7±1.4
2	*Coleus forskohlii*	Lamiaceae	Leaf	58.4±4.6
3	*Asparagus racemosus*	Asparagaceae	Root	56.1±3.7
4	*Centella asiatica*	Mackinlayaceae	Leaf	41.1±1.3
5	*Andrographis paniculata*	Acanthaceae	Leaf	37.3±3.8
6	*Ocimum basilicum*	Lamiaceae	Leaf	27.1±1.3
7	*Ocimum gratissimum*	Lamiaceae	Leaf	37.8±1.3
8	*Coleus blumei*	Lamiaceae	Leaf	38.1±3.8
9	*Coleus solenostemon*	Lamiaceae	Leaf	158.5±1.3
10	*Leucas aspera*	Lamiaceae	Leaf	12.1±0.9
11	*Terminalia arjuna*	Combretaceae	Bark	19.0±0.7
12	*Acorus calamus*	Acoraceae	Root	57.0±1.4
13	*Glycyrrhiza glabra*	Fabaceae	Root	24.9±2.8
14	*Nyctanthes arbortristis*	Oleaceae	Leaf	14.1±0.3
15	*Emblica officinalis*	Euphorbiaceae	Fruit	616.4±0.9
16	*Terminalia bellerica*	Combretaceae	Fruit	63.1±2.2
17	*Terminalia chebula*	Combretaceae	Fruit	83.1±1.1
18	*Withania somnifera*	Solanaceae	Root	120.0±2.5
19	*Evolvulus alsinoides*	Convolvulaceae	Leaf	132±0.8
20	*Tridox procumbens*	Asteraceae	Leaf	89±0.8
21	BHA			17.5±1.9
22	Ascorbic acid			7.3±0.3

Table 2: Antifungal activity of herbs against *R. Stolonifer*

Sample	Fungal growth inhibition (%)
1	68.07
2	38.26
3	13.85
4	15.01
5	24.84
6	14.56
7	26.91
8	20.43
9	10.2
10	70.06
11	48.12
12	23.91
13	65.2
14	61.9
15	18.79
16	12.6
17	5.72
18	26.55
19	32.16
20	26.2
Benzoic acid	65.9
BHA	60.40

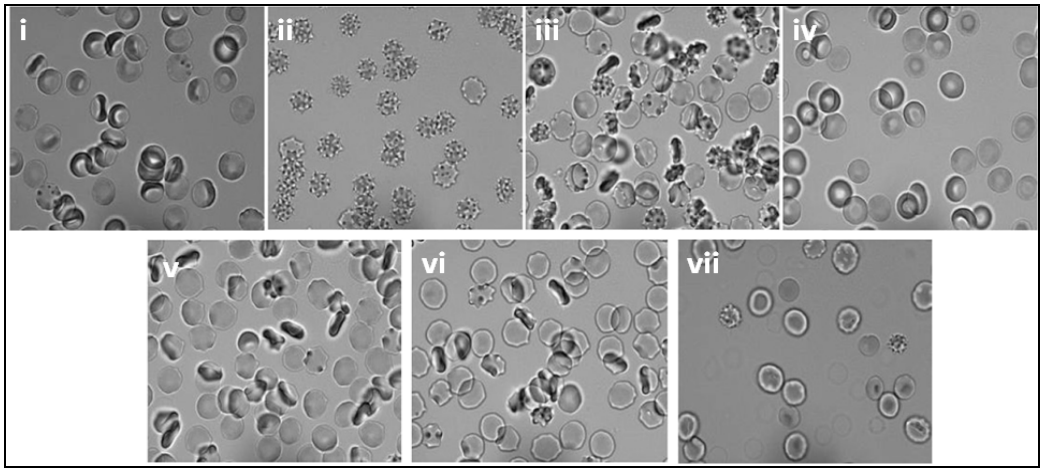

Fig. 1: Optical microscopic evaluation of the erythrocyte morphology. (i) Control–RBCs; (ii) RBCs plus oxidant; (iii) RBCs plus *O. tenuiflorum* extract; (iv) RBCs plus *L. aspera* extract; (v) RBCs plus *T. arjuna* extract; (vi) RBCs plus *G. glabra* extract; (vii) RBCs plus *N. arbortristis* extract at 50 µg/ml and oxidant after 1 h of incubation

RESULTS AND DISCUSSION

In this study, the following was investigated in the selected plant extracts: total phenolic content, cytoprotection of human erythrocytes, DPPH radical scavenging activity and anti-rhizopus activity. The presence of phenolic compounds in herbal extracts has been previously reported [1,3]. High total phenolic content was observed in aqueous extract *O. tenuiflorum*, *L. aspera*, *T. arjuna*, *G. glabra* and *N. arbortristis* (1289, 3837, 372, 2831 and 1892 µg GAE/g respectively) when compared to other plant extracts.

Quantitative antioxidant activity of herbal extracts was evaluated by DPPH radical scavenging [1]. DPPH scavenging model system indicated free radical scavenging ability of the herbal extracts (table 1). The aqueous extracts of *O. tenuiflorum*, *L. aspera*, *T. arjuna*, *G. glabra* and *N. arbortristis* showed highest free radical scavenging ability with IC_{50} of 13.74, 12.13,19.04, 24.9 and 14.14 µg/ml respectively. These results showed the potential electron donating ability of the selected plant aqueous extracts.

The results of cytoprotectivity on erythrocytes on oxidation as indicated in the optical micrographs (fig. 1) showed the protective ability of aqueous extracts of *O. tenuiflorum*, *L. aspera*, *T. arjuna*, *G. glabra* and *N. arbortristis* on erythrocyte membrane protection when compared to other plant extracts. Erythrocytes treated with hydrogen peroxide showed the appearance of echinocytes, indicating damage to the cell membrane. While in plant extracts treated samples, the presence of normal cells can be seen in addition to oxidized cells indicating the protective role of these herbal extracts. Cytoprotective ability of these plant extracts may be attributed to their polyphenolic components.

There are no reports of cytoprotective abilities of polyphenols in these herbal extracts. However, reports are available in the literature from other plant sources [11]. Erythrocytes have been extensively used to study oxidative stress, which represents a simple cell model. Oxidants produce alterations in the erythrocyte membrane as manifested by a decreased cytoskeletal protein content and production of high molecular weight proteins which leads to abnormal erythrocyte shape [12]. Hydrogen peroxide and ascorbate/Fe2+induce an echinocytic type of shape alteration, characterized by protuberances over the cell membrane (Fig.1 i-ii), indicative of oxidative damage. From our results (Fig.1 iii-vii), it is evident that aqueous extracts of some of the selected medicinal plants: *O. tenuiflorum*, *L. aspera*, *T. arjuna*, *G. glabra* and *N. arbortristis* were effective in bringing down the oxidative stress induced erythrocyte cell damage.

Antifungal potential of the herbal extracts was evaluated against *R. stolonifer*. All the twenty extracts showed varying degree of activity. Appreciable inhibition was observed in *O. tenuiflorum*, *L. aspera*, *T. arjuna*, *G. glabra* and *N. arbortristis* extracts (table 2). Although the genus *Ocimum* has frequently been used and the active constituents might be flavonoids [13]. Highest fungal inhibition was observed in *Leucas aspera* which studies have revealed the presence of phenolic compounds in its extract [1]. The correlation co-efficient between *L. aspera* total phenolic content and cytoprotective activity. This suggests the contribution of polyphenols in the plant extracts bioactivity.

The present work demonstrated the antioxidant, cytoprotective and anti-rhizopus activities of the selected plant extracts. The activities exhibited by some of the extracts could be the basis for their alleged health promoting potential. They could serve as new sources of natural antioxidants or nutraceuticals with potential applications in reducing oxidative stress conditions.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

REFERENCES

1. Meghashri S, Vijay Kumar H, Gopal S. Antioxidant properties of a novel flavonoid from leaves of *Leucas aspera*. Food Chem 2010;122:105-10.
2. Esra M, Salih Y. Evaluation of phytochemicals and antioxidant activity of *Ginkgo biloba* from turkey. Pharmacologia 2012;3:113-20.
3. Roselli M, Lentini G, Habtemariam S. Phytochemical, Antioxidant and anti-α-glucosidase activity evaluations of *Bergenia cordifolia*. Phytother Res 2012;26:908-14.
4. Imaida KF, Shivai S, Ohtani T, Nakanish M, Ito K. Promoting activities of butylated hydroxyanisole and butylated hydroxytoulene on 2-stage urinary bladder carcinogenesis and inhibition of γ-glutamyl transpeptidase-positive foci development in the liver of rats. Carcinogenesis 1983;4;895-9.
5. Wong PYY, Kitts DD. Studies on the dual antioxidant and antibacterial properties of parsley (*Petroselinum crispum*) and cilantro (*Coriandum sativum*) extracts. Food Chem 2006;97:505-15.
6. Northover J, Zhou T. Control of rhizopus tor of peaches with postharvest treatments of tebuconazole, fludioxonil and *Pseudomonas syringae*. Can J Plant Path 2002;24:144-53.

7. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1958;18:1199-200.
8. Wagner H, Bladt S. Plant drug analysis: a thin layer chromatography atlas. Springer: Berlin; 1996.
9. Suwalsky M, Orellana P, Avello M, Villena F. Protective effect of *Ugni molinae* Turcz against oxidative damage of human erythrocytes. Food Chem Toxicol 2007;45:130-5.
10. Zhang ZZ, Li YB, Qi L, Wan XC. Antifungal activities of major tea leaf volatile constituents toward *Colletorichum camelliae Massea*. J Agric Food Chem 2006;54:3936-40.
11. Lima CF, Valentao PCR, Andrade PB, Seabra RM, Ferreira MF, Wison CP. Water and methanolic extracts of *Salvia officinalis* protect HepG2 cells from t-BHP induced oxidative damage. Chem Biol Interface 2007;167:107-15.
12. Battistelli M, Sanctis R, De Bellis R, Cucchiarini L, Dacha M, Gobbi P. *Rhodiola rosea* as antioxidant in red blood cells: unstructural and hemolytical behavior. Eur J Histochem 2005;49:243-5.
13. Jyoti Sethi, Sushma Sood, Shashi Seth, Anjana Talwar. Evaluation of hypoglycemic and antioxidant effect of *Ocimum Sanctum*. Indian J Clin Biochem 2004;19:152-5.