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In vitro Antioxidant Activity of Ethanolic Extracts of *Centella asiatica*, *Punica granatum*, *Glycyrrhiza glabra* and *Areca catechu*

M.S. Ashawat, Saraf Shailendra and Saraf Swarnlata
Institute of Pharmacy, Pt. Ravishankar Shukla University,
Raipur (C.G.), 492010, India

Abstract: The present investigation deals with antioxidant activities of the ethanolic extract of *C. asiatica* fresh leaves, *P. granatum* seeds, *G. glabra* dry root and *A. catechu* nuts. The effects of all ethanolic extracts of the herbs were studied via reducing power estimation method. The antioxidant activities of the extracts were compared with standard i.e., of ascorbic acid. The equal amount of extract and ascorbic acid combination antioxidant activity was also studied to know the synergistic effect of chemical and extract in any pharmaceutical and cosmetic formulations. The individual antioxidant activity of *C. asiatica*, *P. granatum*, *G. glabra* and *A. catechu* were found to be 8.23±0.12, 24.35±0.25, 24.25±0.52, 31.31±1.80%, respectively to the standard indicating antioxidant activity to all ethanolic extracts, but catechu showed highest activity ($p<0.05$). The combined antioxidant activity (ascorbic acid with extracts) showed additive synergistic effect as compared to standard i.e., 110.51±0.422, 127.51±0.745, 128.09±0.235, 134.73±0.60, respectively with each extract. These studies may suggest that the combination of chemical with extract as antioxidant can be utilized in pharmaceutical and cosmetic formulation or chemical antioxidant replaced by herbal natural antioxidants.

Key words: *A. catechu*, *C. asiatica*, *P. granatum*, *G. glabra*. reducing powers, antioxidant

INTRODUCTION

Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxides, hydroxy radicals etc. Various environmental exposures such as pollution, tobacco smoke; the sun's ultraviolet light and radiation create free radicals (Hanson *et al*., 2006). Our body also generates free radicals as by-products of burning fuel for energy within the cells, exercising and vanishing off infections. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, which leads the cellular damage. This long-term damage occurs as a result of skin irritations or allergic reactions such as hives and itchy rashes as well as continuous aging of the skin. Naturally occurring antioxidants like alpha carotene, ascorbic acids (Vitamin C), flavone and flavanone have ability to donate electrons and stop free radical chain reactions (Bajpai *et al*., 2005; Tanaka and Masuda, 1998). Many plants, citrus fruits and leafy vegetables as source of ascorbic acid, vitamins E and phenolic compounds, possess the ability to reduce the oxidative damage. This oxidative damage are associated with many diseases like ultra violet radiations induced dermal cancer, cardiovascular disease, cataracts, atherosclerosis, diabetes, arthritis, immune deficiency diseases and ageing (Lee *et al*., 2000).

The L-Ascorbic acid shows the antioxidant activity and due to this activity there are many poly herbal formulations containing L-Ascorbic acid as antioxidant (0.2-4%) are available in market for antiaging, antioxidants, cosmeceuticle in form of lotions, creams, sun care products and shampoos etc. for enhancement of the body's resistance to an assortment of diseases, including infectious disorders and many types of cancer (Choudhuri, 2002).

Corresponding Author: Swarnlata Saraf, Institute of Pharmacy, Pt. Ravishankar Shukla University, Raipur (C.G.), 492010, India Fax: 917712262832

The aim of present investigation was to evaluate the antioxidant activity of different herbal extracts alone or in combination with ideal antioxidant ascorbic acid to replace synthetic chemical compound totally or partially from existing pharmaceutical and cosmetic formulations, which ultimately reduce side effects of chemical after long period of use. The herbs selected for present investigation were leaves of *Centella asiatica*, root of *Glycyrrhiza glabra*, *Punica granatum* seeds and *Areca catechu* nuts. The herbs used in present investigation possesses different activities e.g., antioxidant, antiproliferative, photoprotective, antityrosinase, antiaging, antiinflammatory, antielastase and antimelanogenesis etc. (Nakamura and Yoshikawa, 2007; Kasai *et al*., 2006; Kapoor, 2005; Knaiat, 2000; Schmidt *et al*., 2005; Sudheesh and Vijayalakshimi, 2005).

In the present study antioxidant activity of ethanolic extracts of *C. asiatica*, *P. granatum*, *G. glabra* and *A. catechu* were evaluated using ascorbic acid as standard. Combinations of ascorbic acid to above extracts in equal amount are used to know synergistic activity via reducing power model.

MATERIALS AND METHODS

Materials and Instruments

All the herbs *C. asiatica*, dried roots of *G. glabra*, dried nuts of *A. catechu* and dried seeds of *P. granatum* were procured from authentic vanya aushdhi distributor of Raipur, (C.G.) India. All herbs and part used were authentify from the available herbarium of the Department of Pharmacognosy at Institute of Pharmacy, Pt. R.S. Shukla University, Raipur, (C.G.). Trichloroacetic acid, potassium ferricyanide, ethanol, L-ascorbic acid and ferric chloride all were of analytical grade. Microcentrifuge (RM-12C DX, Remi) and UV spectrophotometer (UV Visible spectrophotometer 1700-Pharmaspec, Shimadzu, Japan) were used for the present study.

Preparation of Extract

Plant materials were cleaned to remove the dirt and extra genus material. All the dried herbal parts roots of *G. glabra*, dried nuts of *A. catechu* and fresh leaves of *C. asiatica*, were made to a coarse powder (particle size ~0.25 mm) using a laboratory mill separately. All the three drugs powder of known quantity (250 g) extracted with hydroalcoholic mixture (1000 mL, 85% v/v) at 60-70°C for 8 h by continuous hot extraction method. The seeds of *P. granatum* (previously dried and minced) were extracted with hydroalcoholic mixture (1000 mL, 85% v/v) using cold maceration process according to Indian pharmacopoeia process M for 8 h (Indian Pharmacopoeia, 1985). To make concentrate and dried extracts (volume reduced to seven times of actual), the solvent was evaporated under reduced pressure (AU 5 psi) at 50±5°C for 5-15 min and extracts were dried and actual percent yields were calculated from the obtained mass and initial powdered drug (Rajpal, 2004).

Antioxidant Activity

The relative reducing activity in terms of antioxidant activity of extracts was determined by using individual extracts (5 mg) as well as its combination with equal amount of ascorbic acid. The extracts and ascorbic acid were dissolved separately in 1.0 mL of deionized water with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 mL). The mixture was incubated at 50°C for 20 min. Aliquots of trichloroacetic acid (2.5 mL, 10% w/v) were added to the mixture and centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared $FeCl_3$ solution (0.5 mL, 0.1%). The absorbance was measured at 700 nm by making 500 μg mL^{-1} extracts aliquot. Increased absorbance of the reaction mixture indicated increased antioxidant activity via reducing power with reference to equal amount of standard ascorbic acid (Rajeshwar *et al*., 2005). Similar procedure was repeated to know combination antioxidant power of extracts with ascorbic acid.

Table 1: Antioxidant activity of ethanolic extracts

Aliquots	Abs. (700 nm)	AOA (%)	Abs. with A.A. (700 nm)	Combination AOA (%)
Ascorbic acid	1.19±0.00	100.00	-	-
C. asiatica	0.09±0.00	8.23±0.12	1.31±0.00	110.51±0.42
P. granatum	0.28±0.00	24.25±0.25	1.51±0.00	27.51±0.74
G. glabra	0.30±0.00	25.25±0.52	1.52±0.00	128.09±0.23
A. catechu	0.37±0.02	31.31±1.80	1.60±0.03	134.73±0.60

AOA = Antioxidant activity; AA = Ascorbic acid; Values = Mean±SD; n = 3; $p<0.05$

RESULTS AND DISCUSSION

The results of prepared extracts revealed that highest yield was obtained in case of *P. granatum* (15.75% w/w) and lowest yield from *A. catechu* (7.5% w/w). Cold maceration method in case of *P. granatum* showed more yield as compared to *A. catechu*, *C. asiatica*, (8.5% w/w) and *G. glabra* (11.5% w/w) using hot extraction method.

From the Table 1 it can be seen that the all-ethanolic extracts showed the antioxidant activity, the highest activity is observed with *A. catechu* (31.31±1.80%), while *C. asiatica* extract showed only 8.27±0.12% as compare to standard. The antioxidant activity of *P. granatum*, *G. glabra* extract showed 24.30±0.25%, 25.25±0.52%, respectively, are comparatively higher than *C. asiatica*, this may be due to the alcoholic soluble phytochemicals such as flavonoids, punicalagin, ellagic acid, glycyrrhzin, presence in the prepared alcoholic extracts of *P. granatum* and *G. glabra*. The highest antioxidant activity of *A. catechu* might be due to presence of the phenolic compounds, tannic and gallic acid contents than other selected plant extracts (Lee and Choi, 1999). The synergistic power of ascorbic acid was observed with combination of equal amount of each extract, which showed additive synergism in case of all the extracts.

CONCLUSION

It was found that all the extracts have the potential to be further developed as effective antioxidant agents for skin cosmetics preparations. These finding can be applicable especially in extensively used herbal cosmetic formulations, where only synthetic chemicals are using as an antioxidant to the skin care products such as skin whitening, antiaging and antiscares agent. These natural antioxidants alone or in combination with synthetic chemical antioxidants may produce multiple benefits on skin. Such natural antioxidant containing formulations can contribute in the management of different kinds of diseases as well as for the management of skin care by reducing side effects after long period use of chemical antioxidants.

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