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Free radical scavenging activity of gossypin and nevadensin: An *in-vitro* evaluation

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ABSTRACT

Objectives: The antioxidant potential of gossypin and nevadensin, two flavonoid compounds, were evaluated by *in vitro* methods.

Materials and Methods: Gossypin, nevadensin, and the reference standard, butylated hydroxyl toluene (BHT), were evaluated for DPPH (1, 1-diphenyl-2-picrylhydrazyl), nitric oxide, superoxide, and hydroxyl radical scavenging activity.

Results: Gossypin and BHT showed the potential for significant DPPH radical inhibition of up to 88.52 and 91.45% at 100 µg/ml concentration. With a 100 µg/ml concentration of gossypin, the *in vitro* nitric oxide, superoxide, and hydroxyl radical scavenging activity was found to be 74.00, 74.22, and 67.15%, respectively; and with 100 µg/ml of BHT the corresponding values were 82.24, 81.76, and 73.03% of inhibition, respectively.

Conclusion: The study results showed that gossypin has significant antioxidant activity.

KEY WORDS: Antioxidant activity, gossypin, nevadensin

Oxygen-derived free radicals such as super oxide anion and hydroxyl radical are cytotoxic and promote tissue injuries. Antioxidants act as a major defense against radical-mediated toxicity by protecting against the damages caused by free radicals.[1] The cellular antioxidant status determines the susceptibility to oxidative damage and is usually altered in response to oxidative stress.[2]

The protection offered by fruits and vegetables against oxidative stress in several diseases has been attributed to various antioxidants and vitamins. Dietary phenolic compounds and flavonoids have generally been considered as non- nutrients and their possible beneficial effect on human health have only recently been recognized. Flavonoids are known to possess anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, neuroprotective, and anticarcinogenic activities.[3,4] Therefore, the search for natural antioxidants of plant origin has gained momentum in recent years. In this work, two flavonoids, gossypin and nevadensin, are studied for their *in vitro* antioxidant activities.

Materials and Methods

The chemicals DPPH (1,1-diphenyl-2-picrylhydrazyl), TBA (thiobarbituric acid), Griess reagent, and NBT (nitroblue tetrazolium) were obtained from Sigma, St. Louis, MO, USA. Trichloroacetic acid (TCA) and potassium superoxides were obtained from Merck, KGOA, Germany. All other chemicals and reagents used were of analytical grade.

DPPH radical scavenging activity

The free radical scavenging activity was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH.[5] A 0.1 mM solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 3.0 ml of control (without the test compound, but with an equivalent amount of methanol), i.e., standard butylated hydroxyl toluene (BHT) at different concentration (25-100 µg/ml), and test solutions at different concentrations (5-100 µg/ml) in different test tubes. Thirty minutes later, the absorbance was measured at 517 nm.

Nitric oxide scavenging activity

Nitric oxide scavenging activity was measured by the spectrophotometry method.[6] Sodium nitroprusside (5 mmol) in phosphate-buffered saline was mixed with a control without the test compound, but with an equivalent amount of methanol. Test solutions at different concentrations (5-100 µg/ml) were dissolved in methanol and incubated at 25°C for 30 min. After

30 min, 1.5 ml of the incubated solution was removed and diluted with 1.5 ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid, and 0.1% naphthyl ethylenediamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of the nitrite with sulphanilamide and the subsequent coupling with naphthylethylene diamine dihydrochloride was measured at 546 nm.

Superoxide scavenging

Superoxide scavenging was carried out using the alkaline dimethyl sulfoxide (DMSO) method.[6] Solid potassium superoxide was allowed to stand in contact with dry DMSO for at least 24 h and the solution was filtered immediately before use; the filtrate (200 µl) was added to 2.8 ml of an aqueous solution containing nitroblue tetrazolium (56 µmol), EDTA (10 µmol), and potassium phosphate buffer (10 mmol, pH 7.4). Test solutions at different concentrations (5-100 µg/ml) were added and absorbance was recorded at 560 nm against the control, in which pure DMSO had been added instead of alkaline DMSO.

Hydroxyl radical scavenging activity

The scavenging capacity for hydroxyl radical was measured according to the modified method.[7] The assay was performed by adding 0.1 ml EDTA, 0.01 ml of $FeCl_3$, 0.1 ml H_2O_2, 0.36 ml of deoxyriboase, 1.0 ml of test solutions (5-100 µg/ml) dissolved in distilled water, 0.33 ml of phosphate buffer (50 mM, pH 7.4), and 0.1 ml of ascorbic acid in sequence. The mixture was then incubated at 37°C for 1 h. A 1.0 ml portion of the incubated mixture was mixed with 1.0 ml of 10% TCA and 1.0 ml of 0.5% TBA to develop the pink chromogen, which was measured at 532 nm.

Statistical analysis

The results are presented as means ± SEM. All parameters were analyzed using Student's *t*-test. $P < 0.05$ was considered significant.

Results

Inhibition of DPPH radical

The potential decrease in the concentration of DPPH radical due to the scavenging ability of gossypin and BHT (reference standard) showed significant free radical scavenging activity: 91.45% and 88.52% of inhibition, respectively, at 100 µg/ml. The IC_{50} (the inhibitory concentration at which there is 50% reduction of free radical) of gossypin was found to be 31 µg/ml. Nevadensin did not show any significant activity [Table 1].

Nitric oxide scavenging activity

The scavenging of nitric oxide by gossypin and BHT was concentration dependent. There was a moderate inhibition of nitric oxide formation, with the maximum inhibition being 74.00 and 82.24% at 100 µg/ml of gossypin and BHT, respectively. Similar activity was not found in the case of nevadensin [Table 1].

Superoxide radical scavenging

A moderate inhibition of the superoxide radical was observed with 100 µg/ml each of gossypin and BHT (74.22 and 81.76% respectively). There was no significant inhibition of superoxide radical by nevadensin [Table 2].

Hydroxyl radical activity

The effect of gossypin and BHT on hydroxyl radical and iron (II)-dependent deoxyribose damage was protected significantly at all concentrations; the percentage of inhibition of hydroxyl radical being 67.15% and 73.03%, respectively, at 100 µg/ml. There was moderate inhibition of hydroxyl radical activity by nevadensin (62.83%) as compared to BHT, which showed 73.03% inhibition at 100 µg/ml concentration [Table 2].

Discussion

Oxidative stress, in which large quantities of reactive oxygen species (ROS) like hydrogen peroxide, superoxide ($^{*}O^{-}_{2}$), hydrogen radical (OH^{-}), singlet oxygen, and nitrogen species are generated, is one of the earliest responses to stress. These ROS have a role in disease and aging in animals.[8] The antioxidative system protects the organism against ROS-induced oxidative damage. There are restrictions on the use of synthetic antioxidants, such as BHT, as they are suspected to be carcinogenic.[9] Natural antioxidants, therefore, have gained importance.

DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm,

Table 1

Free radical scavenging activity of gossypin and nevadensin

Drugs	*Concentration (µgm/ml)*	*DPPH radical inhibition (%)*	*Nitric oxide*
Gossypin	5	10.60 ± 0.2698	42.70 ± 0.5411
	10	17.24 ± 1.396	51.67 ± 0.5457*
	25	54.71 ± 2.191**	62.29 ± 1.0380**
	50	84.29 ± 0.1402***	71.00 ± 0.9290***
	100	88.52 ± 0.3861***	74.00 ± 1.7698***
Nevadensin	5	9.53 ± 0.5543	37.57 ± 0.6910
	10	10.02 ± 1.029	41.28 ± 0.5382
	25	17.74 ± 0.4495	44.18 ± 0.4970
	50	20.99 ± 0.5698	47.24 ± 0.6458*
	100	26.74 ± 1.692	49.11 ± 0.2250*
Butylated hydroxyl toluene	25	86.73 ± 0.3915	77.13 ± 0.6458
	50	88.47 ± 0.1520	79.23 ± 1.7770
	100	91.45 ± 0.1782	82.24 ± 0.4976

Values are mean ± SEM, 6 independent analyses. $P < 0.05$*, $P < 0.01$**, $P < 0.001$*** as compared to standard (Student's *t*-test)

Table 2

Free radical scavenging activity of gossypin and nevadensin

Drugs	*Concentration (µgm/ml)*	*Superoxide inhibition (%)*	*Hydroxyl radical inhibition (%)*
Gossypin	5	35.65 ± 0.9198*	46.99 ± 0.7081*
	10	57.05 ± 1.2561***	52.37 ± 0.5575**
	25	68.70 ± 0.7579***	61.71 ± 0.3296***
	50	71.50 ± 0.8742***	67.15 ± 0.6864***
	100	74.22 ± 0.5889***	79.04 ± 0.6439***
Nevadensin	5	28.56 ± 1.6000	42.83 ± 0.6519
	10	39.61 ± 1.8190	49.36 ± 0.8242*
	25	38.40 ± 1.7762	52.81 ± 0.6752*
	50	38.49 ± 1.8220*	52.83 ± 0.4708**
	100	43.56 ± 1.6551**	62.83 ± 0.4191**
Butylated hydroxyl toluene	25	74.82 ± 0.8156	67.77 ± 0.3100
	50	77.06 ± 0.8905	70.58 ± 0.7873
	100	81.76 ±1.6011	73.03 ± 0.3610

Values are mean ± SEM, 6 independent analyses. $P < 0.05$*, $P < 0.01$**, $P < 0.001$*** as compared to standard (Student's *t*-test)

which is induced by antioxidants. The significant decrease in the concentration of the DPPH radical is due to the scavenging ability of gossypin.

Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reduction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrate ions that can be estimated by use of Greiss reagent. Scavengers of nitric oxide compete with the oxygen, leading to reduced production of nitric oxide.[10] Significant scavenging activity was observed for gossypin.

The potentially reactive hydroxyl radicals can cause oxidative damage to DNA, lipids, and proteins. The effect of gossypin and BHT on the inhibition of free radical-mediated deoxyribose damage was assessed by means of iron (II)-dependent DNA damage assay, which showed significant results.[11]

The flavonoid gossypin has potent antioxidant and free radical scavenging effects in different *in vitro* systems, but nevadensin showed no significant effects as compared to standard BHT. Further work is necessary to elucidate the mechanism involved in the antioxidant activity of gossypin.

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References

1. Mallika J, Shyamala Devi CG. *In vitro* and *in vivo* evaluation of free radical scavenging potential of *Cissus quadranguloris*. Afr J Biomed Res 2005; 8:95-9.
2. Bhor VM, Raghuram N, Sivakami S. Oxidative damage and altered antioxidant enzyme activities in the small intestine of Streptozotocin induced diabetic rats. Int J Biochem Cell Biol 2004;36:89-97.
3. Araceli S, Camen RM, Guillermo RS, Salvador M, Rosa MG. Assessment of the anti-inflammatory activity and free radical scavenger activity of tiliroside. Eur J Pharmacol 2003;461:53-61.
4. Rajnaryana K, Sripalreddy M, Chalavadi MR, Krishna DR. Bioflavonoids classification, pharmacological, Biochemical effects and therapeutic potential. Indian J Pharmacol 2001;33:2-16.
5. Sreejayan N, Rao MN. Free radical scavenging activity of curcuminoids. Drug Res 1996;46:169-71.
6. Madan MP, Raghavan G, Ajay Kumar Singh R, Palpu P. Free radical scavenging potential of *Saussarea costus*. Acta Pharm 2005;55:297-304.
7. Rajeshwar Y, Senthil Kumar GP, Malay AG, Mazumder UK. Studies on *in vitro* antioxidant activities of methanol extract of *Mucuna pruriens* (Fabaceae) sedds. Eur Bull Drug Res 2005;13:131-8.
8. Melov S. Animal models of oxidative stress, aging and therapeutic antioxidant interventions. Int J Biochem Cell Biol 2002;34:1395-400.
9. Singh RP, Chidamara Murthy KN, Jayaprakasha GK. Studies on the antioxidant activity of Pomegranate (*Punica granatum*) Peel and seed extracts using *in vitro* models. J Agric Food Chem 2002;50:81-6.
10. Govindarajan R, Rastogi S, Vijayakumar M, Shirwaikar A, Rawat AK, Mehrotra S, *et al*. Studies on antioxidant activities of *Desmodium gangeticum*. Biol Pharm Bull 2003;26:1424-7.
11. Jornot L, Petersen H, Junod AF. Hyrogen peroxide induced DNA damage is independent of nuclear calcium but dependent on redox active ions. Biochem J 1998;335:854-5.