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Free radical scavenging potential of *Saussarea costus*

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Saussurea costus (Falc.) Lipschitz from the family *Asteraceae* is an important medicinal drug, the roots of which are widely used in folk medicine. The antioxidant activity of the plant has been studied using its ability to scavenge DPPH, nitric oxide, superoxide radicals along with its ability to inhibit lipid peroxidation and GSH oxidation. The 1 mg mL^{-1} extract had antioxidant activity with 85.2% reduction of DPPH and a 72.7% decrease in lipid peroxidation. It showed maximum inhibition of superoxide radical of 66.0%, and 58.4% inhibition of nitric oxide formation. The concentration of chlorogenic acid was 0.027% in the extract of *S. costus*. Thus, the therapeutic activity of the plant may be due to its antioxidant activity, probably as a result of the presence of chlorogenic acid.

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Free radicals are highly reactive species produced in the body during normal metabolic functions or introduced from the environment. These are atoms or groups of atoms that have at least one unpaired electron, which makes them highly reactive. Oxygen, although essential to life, is the source of the potentially damaging free radicals. Antioxidants counteract these cellular by-products, called free radicals, and bind them before they can cause damage. In fact, free radicals are believed to play a role in more than sixty different health conditions, including the aging process, cancer and atherosclerosis (1). Antioxidants work in several ways: they may stop the free radical from forming in the first place, or interrupt an oxidizing chain reaction to minimize the damage caused by free radicals. Reactive oxygen species, especially $^{\bullet}OH$, play a major role in oxidative damage of gastric mucosa in almost all forms of gastric ulcer (2, 3). Increased lipid peroxidation, increased protein carbonyl content and decreased level of endogenous GSH are the characteristic features of $^{\bullet}OH$-mediated oxidative damage of the gastric mucosa during ulceration (3–5). Inhibition of the release of free radicals is a potential strategy to control inflammation and is implicated in the mechanism of action of a number of anti-inflammatory drugs including the representative ones like dexamethasone (6).

Saussurea costus (Falc.) Lipschitz commonly known as 'Kuth' from the family *Asteraceae* is an important medicinal drug. Its roots are widely used as a folk medicine. Several

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studies report that the root of *S. costus* exhibited antimicrobial and antinematodial activity (7–9), hepatoprotective activity (10), antiulcer activity (11) and anti-inflammatory activity (12). The authors reported finding caffeic acid derivatives, *viz.*, chlorogenic acid ([1S-(1α, 3β, 4α, 5α)]-3-[[3-(3,4-dihydroxyphenyl)-1-oxo-2-propenyl]oxy]-1,4,5-trihydroxycyclohexanecarboxylic acid), in *S. costus* for the first time by HPLC (13). Chlorogenic acid exhibited antioxidant activity (14).

Although the plant has been reported to contain caffeic acid derivatives (like syringic acid and chlorogenic acid), the antioxidant activity of the plant has now been studied for the first time using its ability to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH), nitric oxide, superoxide radicals, along with its ability to inhibit lipid peroxidation and GSH oxidation.

EXPERIMENTAL

Animals

Male Sprague – Dawley rats (160–180 g) were purchased from the animal house of the Central Drug Research Institute (Lucknow, India). They were kept in the departmental animal house at 26 ± 2 °C and relative humidity 44–55%, light and dark cycles of 10 and 14 h, respectively, for one week before the experiment. Animals were provided with rodent diet (Amruth, India) and water *ad libitum*. All studies were conducted in accordance with the National Institute of Health »Guide for the Care and Use of Laboratory Animals«. The experimental design was approved by the Institute Ethical Committee.

Randomly selected rats (6 for the control, extract and standard treated each) were fasted overnight and were sacrificed by cervical dislocation, dissected and abdominal cavity was perfused with 0.9% saline. Whole liver was taken out and visible clots were removed and a weighed amount of liver was processed to get 10% homogenate in cold phosphate buffered saline, pH 7.4, using a glass teflon homogenizer, and filtered to get a clear homogenate.

Plant material and extraction

The roots of *Saussurea costus* (*Asteraceae*) were collected from the wild Barsu, Uttarkashi district of Urraranchal (India) during September 2000. The specimen voucher (LWG 91285) is deposited at the National Botanical Research Institute (NBRI, Lucknow, India), herbarium for future reference. Roots of *S. costus* were air-dried and powdered coarsely. The powder obtained (250 g) was macerated with 50% aqueous ethanol (1 L x 3) for 24 h, filtered, concentrated under reduced pressure and lyophilized (Labconco, USA) to get the extract (yield 23.6%).

HPLC analysis

HPLC consisting of a Waters model (Water Corp, USA), equipped with a pump (Waters 515) with a Spherisorb ODS2 column RP – 18 (250 x 4.6 mm, i.d., 5 μm particle size) and a Waters PCM Rheodyne injector with a 25 μL loop was used. Detection was

done at 327 nm using a 2996 PDA detector. Solvent system consisted of acetonitrile (A) and 0.1% phosphoric acid (B) with gradient elution: 0–13 min with 10–22% A, 13–14 min with 22–40% A, isocratic to 14.5 min, 14.5–15 min with 40–10% of A and isocratic with 10% of A to 20 min. Flow rate of 1.5 mL min^{-1} was maintained and 20 μL of the sample was injected. The calibration curve using standard chlorogenic acid was plotted and was found linear in the range of 5–15 μg mL^{-1}.

Lipid peroxidation assay

The degree of lipid peroxidation was evaluated by estimating the thiobarbituric acid-reactive substances (TBARS) using the standard method (15) after minor modifications (16). Briefly, different concentrations of the extract (200–1000 μg mL^{-1}) were added to the liver homogenate. Lipid peroxidation was initiated by adding 100 μL of 15 mmol $FeSO_4$ solution to 3 mL of liver homogenate (final concentration of $FeSO_4$ was 0.5 mmol mL^{-1}). After 30 min, 100 μL of this reaction mixture was placed in a tube containing 1.5 mL of 10% trichloroacetic acid (TCA) and centrifuged after 10 min. The supernatant was separated and mixed with 1.5 mL of 0.67% thiobarbituric acid (TBA) in 50% acetic acid. The mixture was heated in a 100 °C water bath at 85 °C for 30 min to complete the reaction. The intensity of the pink coloured complex was measured at 535 nm in a spectrophotometer (Pharmacia Biotech, India). The TBARS were evaluated from the standard curve (absorption against concentration of tetraethoxypropane) and expressed as nmol TBARS per mg of protein.

Reduced glutathione assay (GSH)

Reduced glutathione was determined by Ellman's method (17). Liver homogenate of different extract concentrations (400–800 μg mL^{-1}) was mixed with 0.5 mL of 5% TCA in 0.1 mmol EDTA. The sample was centrifuged at 2000*g* for 10 min, the supernatant was mixed with 2.5 mL of 0.1 mol L^{-1} phosphate buffer (pH 8) and the colour was developed by adding 100 μL of 0.01% dithionitrobenzoic acid (DTNB). Absorbance was measured at 412 nm (Pharmacia Biotech, USA).

DPPH radical scavenging activity

DPPH scavenging activity was measured by the spectrophotometric method (18). To a methanolic solution of DPPH (100 μmol, 2.95 mL), 0.05 mL of the extract dissolved in methanol was added at different concentrations (500–8000 μg mL^{-1}). An equal volume of methanol served as control. Absorbance was recorded at 517 nm at regular intervals of 30 s for 5 min.

Nitric oxide scavenging

Nitric oxide scavenging activity was measured spectrophotometrically (16). Sodium nitroprusside (5 mmol) in phosphate buffered saline was mixed with different concentrations of the extract (12.5–250 μg mL^{-1}) dissolved in methanol and incubated at 25 °C for 30 min. A control without the test compound but with an equivalent amount of methanol was taken. After 30 min, 1.5 mL of the incubation solution was removed and di-

luted with 1.5 mL of Griess reagent (1% sulphanilamide, 2% phosphoric acid, and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphthylethylene diamine dihydrochloride was measured at 546 nm.

Superoxide scavenging

Superoxide scavenging was carried out using the alkaline dimethyl sulphoxide (DMSO) method (19). 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. 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). Sample extract (1 mL) of various concentrations (30–1500 μg mL^{-1}) was added and the absorbance was recorded at 560 nm against the control in which pure DMSO had been added instead of alkaline DMSO.

Statistical analysis

All the data were presented as the mean ± standard error of the mean and analyzed by the Wilcoxon Sum Rank Test followed by unpaired Student's *t*-test for the possible significant interrelation.

RESULTS AND DISCUSSION

The concentration of chlorogenic acid in *S. costus* extract was found to be 0.027%. The retention time of the chlorogenic acid standard of 6.790 min matches exactly that of the chlorogenic acid in the extract of *S. costus* (retention time 6.765 min, Fig. 1).

S. costus extract caused a dose dependent protection against lipid peroxidation with 72% protection at 1 mg mL^{-1} concentration. Initiation of lipid peroxidation by ferrous sulphate takes place either through ferryl perferryl complex (20) or through •OH radical (21). Ferryl-perferryl complex can also initiate lipid peroxidation on its own in a similar manner as •OH, though it is less reactive than •OH. In iron induced lipid peroxidation, however, role of •OH was not found to be significant (22). The values of TBARS upon incubation with the extract are represented in Table I. The inhibition could be caused by the absence of ferryl-perferryl complex or by scavenging of •OH radical or superoxide radical or by changing the Fe^{3+}/Fe^{2+} ratio or by reducing the rate of conversion of ferrous to ferric ion or by chelating the iron itself (23).

S. costus extract inhibited the oxidation of reduced glutathione in a dose-dependent manner as well (Table II). The importance of thiols, especially of cysteine and glutathione, for lymphocyte function has been known for many years. GSH is a non-enzymic mode of defense against free radicals (17). Glutathione is an important constituent of intracellular protective mechanisms against a number of noxious stimuli, including oxidative stress. It has been reported that the depletion of gastric glutathione by diethyl maleate produced gastric ulceration. On the other hand, the intracellular glutathione seems mainly responsible for protection against gastric cell injury induced by ethanol (24).

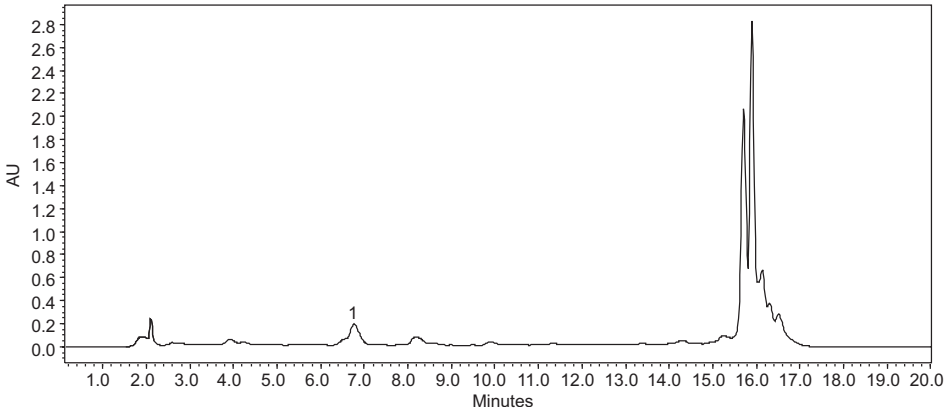

Fig. 1. HPLC fingerprint profile of *Saussurea costus*.
1 – Chlorogenic acid, other peaks not identified.

There was a 85.2% decrease of the DPPH radical at a 1 mg mL^{-1} extract concentration and it was found to be dose dependent (Table III). DPPH is a stable free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. Due to its odd electron, the methanolic solution of DPPH shows a strong absorption band at 517 nm; reduction of the DPPH radicals can be observed by the decrease in absorbance at 517 nm. DPPH radicals react with suitable reducing agents and then electrons become paired off and the solution loses colour stoichometrically with the number of electrons taken up (25). Such reactivity has been widely used to test the ability of compounds/plant extracts to act as free radical scavengers.

There was a moderate inhibition of the superoxide radical, with the maximum inhibition being 66% at 1 mg mL^{-1} extract concentration (Table III). Superoxide radical $O_2^{\bullet-}$ is a highly toxic species, which is generated by numerous biological and photochemical reactions. Both aerobic and anaerobic organisms possess superoxide dismutase enzymes, which catalyze the superoxide radical breakdown. Superoxide dismutase (SOD) is effective in reducing leukocyte adhesion in inflammatory conditions. The potassium superoxide assay was used to measure the superoxide dismutase activity of *S. costus* extract.

Table I. Effect of ethanolic extract of S. costus *on ferrous sulphate induced lipid peroxidation in rat liver homogenate*

Extract concentration (μg mL^{-1})	TBARS (nmol mg^{-1} protein)[a]	Inhibition (%)[a]
Control	2.40 ± 0.15	–
1000	0.67 ± 0.01	72.7 ± 1.38
800	0.89 ± 0.07	62.7 ± 2.45
600	1.20 ± 0.17	50.2 ± 2.0
400	1.58 ± 0.11	34.2 ± 3.2
200	1.68 ± 0.27	30.0 ± 4.2
Tocopherol (10 μmol L^{-1})	0.06 ± 0.01	97.5 ± 4.3

[a] Mean ± SEM, 6 independent analyses.

Table II. Oxidation of GSH in rat liver homogenate by ethanolic extract of S. costus

Extract concentration (μg mL^{-1})	GSH (nmol mg^{-1} protein)[a]	Inhibition (%)[a]
Control	6.27 ± 0.26	–
1000	1.35 ± 0.31	78.4 ± 2.2
800	1.99 ± 0.39	68.2 ± 2.3
600	2.53 ± 0.07	59.5 ± 1.3
400	2.73 ± 0.19	56.5 ± 2.5
200	3.77 ± 0.34	39.9 ± 2.0
Tocopherol (10 μmol L^{-1})	0.08 ± 0.01	98.7 ± 2.2

[a] Mean ± SEM, 6 independent analyses.

Table III. Free radical scavenging activity of S. costus *extract*

Extract concentration (μg mL^{-1})	DPPH	Superoxide inhibition (%)[a]	Nitric oxide
1000	85.2 ± 4.0	66.0 ± 1.6	58.4 ± 4.2
800	77.8 ± 4.2	59.1 ± 2.8	31.4 ± 1.6
600	66.5 ± 5.8	41.0 ± 1.2	19.7 ± 2.4
400	45.1 ± 1.2	32.2 ± 3.2	11.7 ± 1.2
200	33.7 ± 1.9	21.4 ± 2.2	2.6 ± 0.7
Ascorbic acid (100 μmol L^{-1})	94.3 ± 3.9	87.1 ± 2.7	84.3 ± 4.2

[a] Mean ± SEM, 6 independent analyses.

There was a moderate inhibition of the nitric oxide formation with the maximum inhibition 58.4% achieved with 1 mg mL^{-1} extract concentration (Table III). Incubation of solutions of sodium nitroprusside in phosphate buffer saline (PBS) at 25 °C for 2 h resulted in linear time-dependent nitrite production, which was reduced by the *S. costus* extract. The scavenging of nitric oxide by the extract was concentration-dependent.

The antioxidant activity of the extract can be partly justified by the presence of 0.027% chlorogenic acid.

Since *S. costus* shows potent antioxidant activity, its use in the traditional systems of medicine for its anti-inflammatory and anti-ulcer activity may in part be due to its free radical scavenging ability. Since chlorogenic acid present in this plant is a potent antioxidant it might be responsible for the activity exhibited by the extract.

CONCLUSIONS

Further activity guided isolation and characterization of the *S. costus* extract is in progress to identify the full composition of the extract and the exact compound(s) responsible for the antioxidant activity. Chlorogenic acid can be used as a marker component in quality evaluation/standardization and also in differentiating the roots of *S. costus*.

REFERENCES

1. B. N. Ames, M. K. Shigenaga and T. M. Hagen, Oxidants, antioxidants and the degenerative diseases of aging, *Proc. Natl. Acad. Sci. USA*. **90** (1993) 7915–7922.
2. P. S. Phull, C. J. Green and M. R. Jacyna, A radical view of stomach: the role of oxygen-derived free radicals in gastroduodenal disease, *Am. J. Gastroenterol. Hepatol*. **7** (1995) 265–274.
3. D. Das, D. Bandyopadhyay, M. Bhattacharjee and R. K. Banerjee, Hydroxyl radical is the major causative factor in stress-induced gastric ulceration, *Free Radical Biol. Med*. **23** (1997) 8–18.
4. D. Bandyopadhyay, K. Biswas, U. Bandyopadhyay, R. J. Reiter and R. K. Banerjee, Melatonin protects against stress-induced gastric lesions by scavenging the hydroxyl radical, *J. Pineal Res.* **29** (2000) 143–151.
5. M. Bhattacharjee, S. Bhattacharjee, A. Gupta and R. K. Banerjee, Critical role of endogenous gastric peroxidase in controlling oxidative damage in *H. pylori* mediated and nonmediated gastric ulcer, *Free Rad. Biol. Med*. **32** (2000) 731–743.
6. E. Bourke and P. N. Moynagh, Antiinflammatory effects of glucocorticoids in brain cells, independent of NF-kappa B, *J. Immunol.* **163** (1999) 2113–2119.
7. M. S. Akhtar and S. Riffat, Field trail of *Saussurea lappa* roots against nematodes and *Nigella sativa* seeds against cestodes in children, *J. Pakistan Med. Assoc.* **41** (1991) 185–187.
8. S. N. Naik, A. Kumar, R. C. Maheshwari, M. B. Guddewar, R. Chandra and B. Kumar, Pesticidal properties of sub-critically extracted plant essential oils against storage pest Tribolium castaneum (Herbst), *Indian Perfum.* **39** (1995) 171–176.
9. S. Farooq and G. K. Pathak, A comparative study of 'in vitro' antimicrobial activity of total solvent extracts of some medicinal plants with Asawa and Aarishta preparation, *J. Non-Timber Forest Prod.* **5** (1998) 79–81.
10. H. Okugawa, R. Ueda, K. Matsumoto, K. Kawanishi and A. Kato, Effect of dehydrocostus lactone and costunolide from *Saussurea* root on the central nervous system in mice, *Phytomedicine* **3** (1996) 147–153.
11. J. Yamahara, M. Kobayashi, K. Miki, M. Kozuka, T. Sawada and H. Fujimura, Cholagogic and antiulcer effect of *Saussurea* radix and its components, *Chem. Pharm. Bull*. **33** (1985) 1285–1288.
12. M. Jin, H. J. Lee, J. H. Ryu and K. S. Chung, Inhibition of LPS-induced NO production and NF--kappa B activation by a sesquiterpene from *Saussurea lappa*, *Arch. Pharm. Res.* **23** (2000) 54–58.
13. M. M. Pandey, R. Govindrajan, A. K. S. Rawat, Y. P. S. Pangtey and S. Mehrotra, High performance liquid chromatographic method for quantitative estimation of an antioxidant principle chlorogenic acid in *Saussurea costus* and *Arctium lappa*, *J. Nat. Prod. Sci.* **10** (2004) 40–42.
14. M. Shafiee, M. A. Carbonnart, J. B. Huart, B. Descomps and C. L. Heger, Synergistic antioxidative properties of phenolics from natural origin towards low-density lipoproteins depend on oxidation system, *J. Med. Food* **5** (2002) 69–78.
15. H. Okhawa, N. Ohishi and K. Yagi, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, *Anal. Biochem*. **95** (1979) 351–355.
16. R. Govindarajan, S. Rastogi, M. Vijayakumar, A. K. S. Rawat, A. Shirwaikar, S. Mehrotra and P. Pushpangadan, Studies on antioxidant activities of *Desmodium gangeticum*, *Biol. Pharm. Bull*. **26** (2003) 1424–1427.
17. Y. B. Tripathi and M. Sharma, Comparison of the antioxidant action of the alcoholic extract of *Rubia cordifolia* with rubiadin, *Indian J. Biochem. Biophys*. **35** (1998) 313–316.
18. N. Sreejayan and M. N. A. Rao, Free radical scavenging activity of curcuminoids, *Drug Res.* **46** (1996) 169–171.
19. L. E. A. Henry, B. Halliwell and D. O. Hall, The superoxide dismutase activity of various photosynthetic organisms measured by a new and rapid assay technique, *FEBS Lett*. **66** (1976) 303–307.

20. J. M. C. Gutteridge, Age pigments and free radicals: fluorescent lipid complexes formed by iron and copper containing proteins, *Biochim. Biophys. Acta* **834** (1985) 144.
21. R Govindarajan, M. Vijayakumar and P. Pushpangadan, Antioxidant approach to disease management and the role of 'Rasayana' herbs of Ayurved, *J. Ethnopharmacol.* **99** (2005) 165–178.
22. E. Graf, J. R. Mahoney, R. G. Bryant and J. W. Eaton, Iron-catalyzed hydroxy free radical formation: stringent requirement for free iron co-ordination site, *J. Biol. Chem.* **259** (1984) 3620–3623.
23. J. M. Braugghler, C. A. Duncan and L. R. Chase, The involvement of iron in lipid peroxidation. Importance of ferrous to ferric ratio in initiation, *J. Biol. Chem.* **261** (1986) 10282.
24. R. Govindarajan, M. Vijayakumar, A. K. S. Rawat and S. Mehrotra, Free radical scavenging potential of *Picrorhiza kurrooa* Royle ex Benth., *Indian J. Exp. Biol.* **41** (2003) 875–879.
25. M. Blois, Antioxidant determinations by the use of stable free radical, *Nature* **26** (1958) 1199.

S A Ž E T A K

Sposobnost hvatanja slobodnih radikala biljke *Saussarea costus*

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Saussurea costus (Falc.) Lipschitz *(Asteraceae)* važna je ljekovita biljka čiji se korijen upotrebljava u narodnoj medicini. U ovom radu proučavano je antioksidativno djelovanje te biljke. Praćena je sposobnost vezanja DPPH, oksida dušika, superoksidnih radikala i inhibicije peroksidacije lipida i oksidacije GSH. 1 mg mL^{-1} ekstrakta reducira 85,2% DPPH i za 72,7% smanjuje peroksidaciju lipida. Maksimalna inhibicija superoksidnih radikala bila je 66,0%, a inhibicija stvaranja oksida dušika 58,4%. Koncentracija klorogenske kiseline u ekstraktu *S. costus* je 0,027%. Smatra se da je upravo prisutnost te kiseline presudno za antioksidativno djelovanje.

Keywords: Saussurea costus (Asteraceae), antioksidans, peroksidacija lipida, klorogenska kiselina

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