Determination of In-Vitro Antioxidant Activity of Kasmard (*Cassia Sophera* Linn) Leaves

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Summary

The antioxidant activities of aqueous, ethanol, and Pet. ether extracts of the leaves of *cassia sophera* Linn, were determined by the thiocyanate method using the linoleic acid emulsion. Ether extract was the most effective antioxidant among the extracts. Like antioxidant activity, the scavenging power of ether extract was the highest and aqueous extract was the lowest. The results obtained in the present study indicate that the leaves of *cassia sophera* are a potential source of natural antioxidants. In addition, we could suggest that although the scavenging activity of an extract may be an indicator of its potential antioxidant activity, it is important to evaluate this plant in detail with other methods. Phytochemical isolation of the ether extract for active antioxidant moiety will be the further scope for the study.

Key Words: Antioxidant activity, *cassia sophera*, DPPH, Thiocyanate method

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Introduction

Reactive oxygen species (ROS), sometimes called active oxygen species, are various forms of activated oxygen, which include free radicals such as superoxide ions (O_2^-) and hydroxyl radicals (OH), as well as non free-radical species such as hydrogen peroxide (H_2O_2) (1,2). In living organisms various ROSs can be formed in different ways, including normal aerobic respiration, stimulated polymorphonuclear leukocytes and macrophages, and peroxisomes. Exogenous sources of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents, and pesticides (3–5). Free radicals can cause lipid peroxidation in foods leading to their deterioration (6, 7). In addition, reactive oxygen species have been implicated in more than 100 diseases, including malaria, acquired immunodeficiency syndrome, heart diseases, stroke, atherosclerosis, diabetes and cancer [8-11]. When produced in excess, ROSs can cause tissue injury. However, tissue injury can itself cause ROS generation[12]. However natural antioxidant mechanism can be inefficient and hence dietary intake of antioxidant compounds is necessary (13, 14). There are some synthetic antioxidant compounds such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), commonly used in processed foods. However, it has been discovered that these compounds have some side effects (15, 16). In addition, it has been suggested that there is an inverse relationship between dietary intake of antioxidant rich foods and the incidence of human disease (17). Therefore, research for the determination of the natural antioxidants source is important.

Cassia sophera Linn. (Caesalpiniaceae) is known as “Kasmard” in Ayurvedic literature it means cough suppressant (Kas - cough; Mard - to protect). (18) In folk literature it is used in asthma as expectorant, GIT disorder, and rheumatic disorders. It is reported to be used in homoeopathy; decoction of plant is used as antidiuretic. The juice made with the paste of sandal wood and lime juice is considered specific for ring worm. An infusion of leaves is given with sugar in jaundice, and in sub acute stage of gonorrhea. It is used to as febrifuge in rheumatic and inflammatory fever. However it is also used in some immunomodulatory preparations of homeopathy and Ayurveda (19, 20). The purpose of this study is the determination of antioxidant activities of various extracts of leaves of *Cassia sophera* Linn.

Material and Methods

Chemicals

Chemicals used in this study were 1, 1-diphenyl-2-picrylhydrazyl (DPPH), potassium ferricyanide, sodium nitrite, trichloroacetic acid, Folin-Ciocalteu reagent, butylated hydroxy anisole (BHA), ascorbic acid (Merck [India]), Gallic acid, linoleic acid (Sigma), α- tocopherol. All reagents used for the experiments were of analytical grade (AR).

Collection of plant material: Leaves were collected from Khandobachiwadi of Pune region in Maharashtra (India) in August 2004. Plant was identified and authenticated from expert botanist in the Botanical survey of India, Pune, India.

Preparation of Extracts: 20-gram dried sample (leaves of *Cassia sophera* Linn.) was chopped into small parts in a blender and then extracted with 450 ml of boiled water by stirring for 30 min followed by filtration concentrated with rota evaporator. The filtrate was freeze-dried. For ethanol extract 20g of dried and chopped leaves was extracted with 450 ml ethanol by stirring for 6 hours. In the ether extraction, the same amount of sample was extracted with ether in a soxhlet apparatus until extraction solvents become colorless. Both of the extracts were filtered and evaporated to dryness in vacuum.

Estimation of total phenolics: The total phenolic contents of ethanol extract was determined with Folin-Ciocalteu reagent according to Slinard & Singleton (21) and slightly modified. The stock solution of extract 1mg/ml in water was prepared. From the stock solution, 5 ml was transferred to a 25 ml volumetric flask and made up with distilled water. Out of this 5 ml of sample and 2 ml of standard was taken in 25-ml volumetric flask, to this 10 ml of distilled water, and 2ml of phenol reagent (20%v/v) was added, and then the volume was made up with 29% sodium bicarbonate. The mixture was kept in the dark for 20 min. and the absorbance was read at 760 nm. The total phenolic content was calculated as gallic acid and expressed as percent of gallic acid detected. Standard used was gallic acid.

Antioxidant assay

Thiocyanate method: Antioxidant activity was determined by the thiocyanate method (22). Each (1000µg/ml) sample of extract in 0.5 ml of distilled water was mixed with 2.5 ml of linoleic acid emulsion (0.02M) and 2 ml phosphate buffer (0.04M, pH 7.0) in a test tube and incubated in darkness at 37ºC. The amount of peroxide was determined by reading absorbance at 500 nm after coloring with $FeCl_2$ and thiocyanate at intervals during incubation. α - Tocopherol was used as standard antioxidant.

Free radical scavenging activity using DPPH radical: The free-radical scavenging activities of *C. Sophera* extracts were measured by decrease in the absorbance of methanol solution of DPPH. A stock solution of DPPH (33 mg in 1 l) was prepared in methanol, which gave initial absorbance of 0.8, and 5ml of this stock solution was added to 1ml of *C. Sophera* different extracts solution at concentrations (50-500 mg). After 30 min, absorbance was measured at 517 nm. Antiradical activity was calculated as % inhibition from the given formula:

$$\% \text{ Anti-radical activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Nitric oxide scavenging activity: The nitric oxide scavenging property of the extracts showed could not assessed, as the color components of the extracts interfering with the measurement of the chromophore formed in the reaction mixture. Hence procedure as well as result has not been mentioned.

Results and Discussion

In this report the percentage of total phenolics was calculated and found to be 0.92 – 1.10 % as gallic acid in *cassia sophera* leaves. In the present study, antioxidant activity was determined by the thiocyanate method reveals that the amount of peroxides formed in emulsion during incubation was determined spectrophotometrically by measuring absorbance at 500 nm. High absorbance is an indication of high concentrations of formed peroxides.

Ethanol extract (1.14) had higher activity than aqueous extract (0.87), but the difference was not statistically significant. Nevertheless, the most effective antioxidant activity was shown by ether extract (0.07), hence it was able to delayed peroxidation for 30 hours (Figure 1). It was also interesting to find that ether extract had antioxidant activity near to α-tocopherol (standard) so can be considered as alternative source.

The DPPH anti- radical scavenging activity involves reaction of specific antioxidant with a stable free radical 2, 2-diphenyl-1- picryl-hydrazyl DPPH. As a result, there was reduction of DPPH concentration by antioxidant, which decreases the optical absorbance of DPPH; this is detected by spectrophotometer at 517 nm.

Table.1 expresses a significant decrease in the concentration of DPPH radical (percentage inhibition) due to the scavenging ability of all extracts of *Cassia Sophera*. The scavenging effect of different extracts on the DPPH radical decreased in the order Pet Ether > Ethanolic > aqueous and were 71.87%, 63.26 %, and 24.27%, respectively, at a concentration of 1000

mg/ml. These results indicated that all extracts have a noticeable effect on scavenging the free radicals. All of the extracts of *cassia sophera* showed less activity when compared with that of BHA and Ascorbic acid in case of DPPH scavenging effect.

Table 1. Antioxidant activities of *cassia sophera* leaves.

No.	Compound	Concentration (µg/ml)	DPPH radical scavenging activity (% inhibition)	Thiocyanate Method (Absorbance after 30 Hrs)
1.	Aqueous extract	1000	71.87%	0.8697
2.	Ethanolic extract	1000	63.26%	1.1453
3.	Pet. Ether extract	1000	24.27%	0.0748
4.	α-Tocopherol	500	--	0.0371
5.	BHA	25	60.42%	--
6.	Ascorbic acid	15	49.89%	--

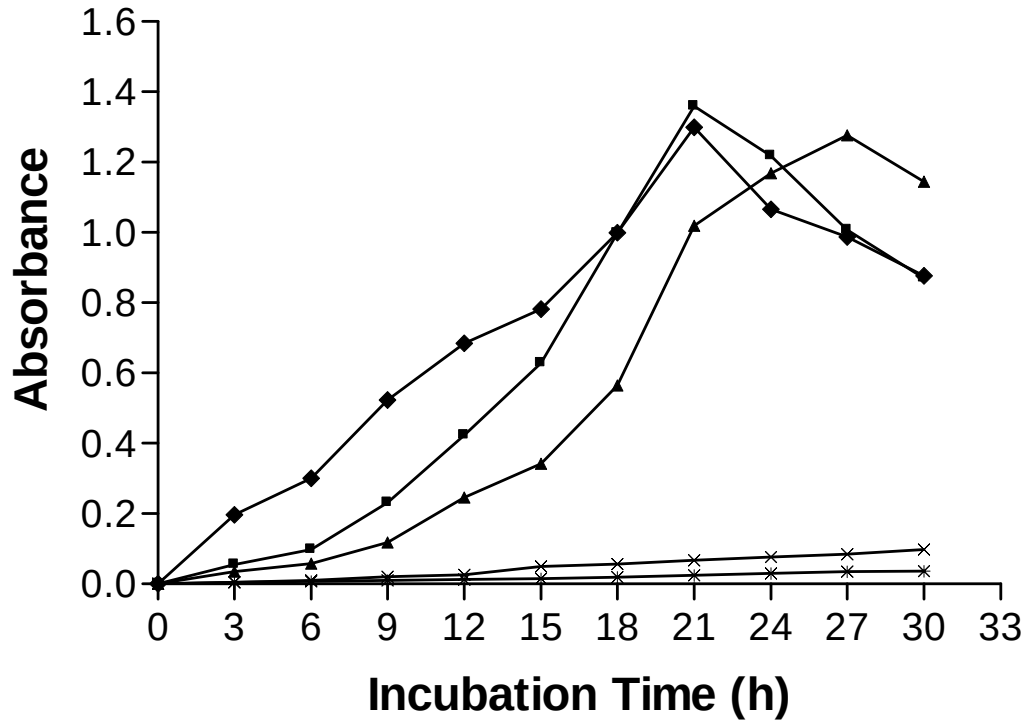

Figure 1: Antioxidant activities of dried ether, ethanol, and aqueous extract of the leaves of *Cassia sophera*. In each there was 500 µg of indicated dried extracts. (■ - Control; ◆- Aqueous; ✕ - Pet. ether; ▲ - ethanol; ✳- STD: α-tocopherol).

References

1. Halliwell B. How to characterize an antioxidant: an update. *Biochem. Soc. Symp,* 1995. 61: 73-101.
2. Squadriato Gl.; Pelor WA. Free Radical Oxidative chemistry of nitric oxide: The roles of superoxide, peroxynitrite, and carbon dioxide. *Biology and Medicine* 1998, 25: 392-403.
3. Halliwell B. Gutteridge JM. Free radicals in biology and medicine. Clarendon Press Oxford, 23- 30, 1989.
4. Davies K J A. Oxidative stress the paradox of aerobic life. *Biochem. Symp.* 1994, 61: 1-34.
5. Robinson EE. Maxwell SRJ. Thorpe GHG. An investigation of the antioxidant activity of black tea using enhanced chemiluminescence. *Free Rad. Res.* 1997, 26: 291-302.
6. Sasaki S. Ohta T. Decker EA. Antioxidant activity of water soluble fractions of salmon spermary tissue. J. Agric. Food Chem. 1996, 44: 1682-1686.
7. Miller NJ. Diplock AT. Rice-Evans CA. Evaluation of the total antioxidant activity as a marker of the deterioration of apple juice on storage. *J. Agric. Food Chem* 1995, 43:1794-1801,.
8. Tanizawa H.*et al* Studies on natural antioxidants in citrus species I. Determination of antioxidative activities of citrus fruits. *Chem. Pharm. Bull.* 1992, 40: 1940-1942,
9. Hertog MGL. Feskens EJM. Hollman PCH. Katan MB.Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: The zupthen elderly study. *The Lancet* 1993, 342: 1007- 1014.
10. Alho H. Leinonen J. Total antioxidant activity measured by chemiluminescence method. *Methods in Enzymology*, 1999, 299: 3-15.
11. Duh P-D. Antioxidant activity of Burdock: Its scavenging effect on free-radical and active oxygen. *JAOCS*, 1998:75, 455- 463,
12. Auroma OI. Free radicals, oxidative stress, and antioxidants in human health and disease *JAOCS*, 1998:75: 199-212.
13. Halliwel B. Free radicals, antioxidants and human disease: Curiosity, cause or consequence. The Lancet 1994, 344: 721-724.
14. Terao J. *et al* Protective effect of epicatechin, epicatechin gallate, and quercetin on lipid peroxidation in phospholipid bilayers. *Arch. Biochem. Biophys.* 308: 278-284, 1994.
15. Branien AL. Toxicoly and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. *JAOCS,* 1975, 52: 59-63.

16. Ito N. *et al* Carcinogenicity of butylated hydroxyanisole in F344 rats. *J Natl. Cancer Inst.* 1983, 70: 343-347.
17. Rice-Evans *et al*. Why do we expect carotenoids to be antioxidants in vivo? *Free Rad. Res*1997. 26: 381-398.
18. Kirtikar KR, and Basu BD, Indian medicinal plants, Dehradun India. II edition 1935, Vol. II reprint- 1999, international book distributors, Page no. 863.
19. Oyaizu M: Studies on product of browning reaction prepared from glucose amine. *Jpn J Nutr* 1986, 307–315.
20. Nadakarni KM Indian material medica Vol.- I, Popular prakashan, mumbai, page no. 291.
21. Slinard K, Singleton VL, Total phenol analysis automation and comparison with manual methods. *Am J Enol Vitic* 1977, 49–55.
22. Yen GH; Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agric. Food Chem*. 43: 27-32, 1995.
23. Sreejayan, Rao MNA, Nitric oxide scavenging by curcuminoids. *J Pharm Pharmacol* 1997, 49: 105–107.