Knowledge Consortium of Gujarat
Department of Higher Education - Government of Gujarat
Journal of Science - ISSN : 2320-0006

Continuous issue-8 | August – September 2016

COMPARATIVE STUDY OF ANTIOXIDANT PROPERTIES OF CINNAMON AND KOKUM IN AQUEOUS AND METHANOLIC EXTRACTS

Abstract

Antioxidants are compounds that help to maintain the levels of reactive oxygen species which in return promote healthy life. There are 2 types of antioxidants enzymatic (catalase, superoxide dismutase and glutathione peroxidase) and non enzymatic (Vitamin C, Vitamin E). Taking a diet rich in antioxidants has always helped in fighting chronic diseases. Researches have promoted the use of natural antioxidants in diet hence extensive research has been carried in isolating compounds with antioxidant properties from plants. There are various extraction medium used which various solved like ether, ethanol, hexane, methanol, water etc. In the present study a comparative analysis of antioxidant properties of two commonly used spices cinnamon and Kokum was carried out using aqueous and methanolic extracts. Methanolic fraction showed higher antioxidant properties measured as total phenol, DPPH, FRAP and ABTS. Cinnamon had higher antioxidant undex compared to Kokum.

Introduction

Plant medicines are the first line of defence in maintaining health and preventing diseases. Approximately 72,000 plant species were having medicinal properties of which India recognizes more than 3,000 plant species having medicinal values [1]. Extensive research on plants, particularly medicinal plants, has been carried out to evaluate their antioxidant activity. Intake of foods rich in natural antioxidants lowers the risks of degenerative diseases. Antioxidants from plants were studied to develop natural antioxidant formulations for food, cosmetic, and other applications [2].

Antioxidants can scavenge free radicals and help to decrease the oxidative stress induced damage to cells. Antioxidants are able to neutralize free radicals before they damage DNA. Antioxidants are most important for maintaining optimal cellular health and well-being. Plant foods contain antioxidants mainly in the form of polyphenol as well as certain vitamins, minerals and pigments. Flavonoid and non-flavonoid polyphenol compounds, including ascorbic acid, β- carotene, vitamin –E and selenium are powerful antioxidants. Oxidation reactions produce free radicals which propagate and damage the cells. An antioxidant slows down this process and prevents the oxidation of other molecules. It also prevents oxidative stress and the other chronic disease conditions [7].

Plant chemicals are divided into three major classes: terpenoids, phenolic metabolites, and alkaloids. Phenolic compounds are important for dietary applications and very extensive researched area. Phenolic compounds include polyphenols (hydrolyzable and condensed tannins), phenolic acids, and flavonoids. These types of compounds have been used as antioxidants by humans [2].

Finding new and safe antioxidants from natural plants is of great interest as they have major applications in functional foods, natural antioxidants and neutraceuticals. Different techniques are used to extract

antioxidants from plant materials such as maceration, ultra-sound assisted extraction supercritical fluid extraction, Soxhlet extraction and subcritical water extraction. Extraction and antioxidant activities are not dependent on the extraction method but also on the solvents used for extraction [2].

The presence of different antioxidant compounds with various chemical characteristics is soluble in particular polar solvents. Polar solvents are used for recovering polyphenols from plant materials. The most suitable solvents are aqueous mixtures containing acetone, ethanol, methanol and ethyl acetate. Ethanol known as a good solvent for polyphenol extraction. Methanol solvent found to be more efficient in extraction of lower molecular weight polyphenols whereas aqueous acetone solvent is good for extraction of higher molecular weight flavanols [2].

Since ancient times herbs and spices have been used to enhance or improve the flavour of food due to their sensory properties and also as preservative agents. However, most of their potential health promoting properties has received little attention. Researches has shown culinary herbs and spices could be a dietary source of bioactive polyphenols, which influents the study of their phenolic composition and antioxidant properties. Many culinary herbs and spices are known for their beneficial effects for human health, including digestive stimulant, anti-inflammatory, antimicrobial, antioxidant and anti-carcinogenic activities [3].

Cinnamon is obtained from the inner bark of various species of the genus Cinnamomum. Chinese cinnamon (Cinnamomum cassia or Cinnamomum aromaticum) from China and Southern and Eastern Asia contains high level of coumarin, a harmful molecule. Ceylon cinnamon (Cinnamomum zeylanicum or Cinnamomum verum) from Sri Lanka and Madagascar contains only traces of coumarin [4].

Cinnamon contains different types of active compounds which includes cinnamaldehyde, cinnamate, cinnamic acid, and numerous essential oils such as trans-cinnamaldehyde, cinnamyl acetate, eugenol, L-bornyl acetate, E-nerolidol, α-cubebene, α-terpineol, L-borneol, caryophyllene oxide, b-caryophyllene, terpinolene and α-thujene [5] .

Cinnamon act as a coagulant to improve the health of the colon by reducing the risk of colon cancer, increases blood circulation and advances tissue regeneration. Essential oils and other constituents of cinnamon also have important activities including antimicrobial, antifungal, antioxidant, antidiabetic, anti-inflammatory, insecticidal, antitermitic, nematicidal, mosquito larvicidal, antimycotic and anticancer agent [5].

Kokum is a tropical evergreen tree and found at an altitude of about 800 meters from sea level. It is a slender tree with drooping branches and grows to a height of 15-20m and the canopy is dense with green leaves. It is a native of the Western Ghats region of India. It is distributed throughout Konkan, Goa, North & south Karnataka, North Malabar, Coorg & Wynad as well as in West Bengal and Assam [6].

Kokum fruit contains active components which include hydroxy citric acid, anthocyanins and a poly-isoprenylated benzophenone derivative and garcinol. Hydroxyl citric acid found to be a potent effector as metabolic regulator for obesity and lipid abnormalities. Kokum contains good amount of B-complex vitamins and minerals like potassium, manganese and magnesium which helps in controlling blood pressure and shows protection against stroke and coronary heart diseases. This fruit has been used to prevent digestive problems such as acidity, indigestion, flatulence and constipation. Kokum fruit contains

important activities including antioxidants, chelating, anti-cancer, anti-fungal, anti-inflammatory, antibacterial, cardio protective and anti-ulcer activities [6].

In present study our objective was to evaluate and compare the antioxidant properties of cinnamon and kokum using aqueous and methanolic extracts.

Methods and materials

The research work was carried out at Jashbhai Khodabhai Patel, P.G.Department of Home Science, Sardar Patel University, V.V.Nagar, Anand.

Chemicals used:

Trolox, Folin cio-calteu, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Rutin, 2,4,6- Tris (2-pyridyl)-s-triazine (TPTZ), Gallic acid and ABTS were purchased from Sigma- Aldrich Ltd. (India).

Sample extraction:

Preparation of sample for total phenol, flavonoid and total antioxidant capacity. 5 gm of dried ground sample were homogenized using blender and the homogenized samples were extracted using 80% acidified methanol (pH-2.0) in shaker for 30 minutes. The extracts were centrifuged at 7000 rpm for 10 minutes and supernatant was collected. The residues were further extracted by adding the same solvent and the extraction procedure was repeated for three times. Volume made to a round number of collected extracts and sample were stored at -20°C and used for total phenol, flavonoid and antioxidant activity analysis.

Total Phenol content assay:

Total phenol content was measured by the spectrophotometric method. Aliquots of sample were taken and to this Folin-ciocalteu reagent (50% v/v with D/W) was added to make up the volume to 0.55ml. All the tubes were vortexed (Gilson Ltd.) and then add 10 ml of 7.5gm% Sodium Carbonate in each tube and allowed to incubate for 1 hour at 37ºC. Then absorbance was measured at 750 nm. Gallic acid was used as a standard. Total Phenol content was expressed as Gallic acid Equivalent [7].

Flavonoid assay:

The total flavonoid content was measured by using colorimetric assay. Sample aliquots were taken and volume was made up to 5 ml with distilled water. Then add 0.3 ml of 5% Sodium Nitrite was added. After 5 min, 0.6 ml of 10% Alcl3 was added. After 6 min, 2 ml of 1N sodium hydroxide was added to the mixture. Then add of 2.1ml of distilled water the solution was mixed well and the intensity of pink colour was measured at 510 nm against distilled water as a blank. Rutin was used as a standard and results are expressed as Rutin equivalent [7].

Ferric Reducing Antioxidant Power assay:

Total antioxidant capacity of extracts was determined by using the method of Benzie and Strain, (1996). Suitable aliquot of sample was taken and volume was made 300 µl with distilled water and then add 1.8 ml of freshly prepared FRAP reagent (Acetate buffer (pH- 3.6) + TPTZ solution+ 20 mM Ferric chloride) was

added and vortexed. After incubation of 10 minutes at 37°C, the absorbance was read at 593nm against distilled water as a blank. Trolox was used as a standard and results are expressed as trolox equivalent [7].

DPPH radical scavenging activity assay:

1, 1- diphenyl, 2-picrylhydrazyl (DPPH) scavenging activity was measured by the spectrophotometric method. Solution of DPPH in methanol was prepared freshly. 3 ml of DPPH solution was mixed with the sample aliquots and incubated for 20 minutes at 37°C. Absorbance was measured at 517 nm against methanol as blank. Trolox was used as a standard and results are expressed as trolox equivalent [7].

ABTS radical scavenging activity assay:

ABTS radical scavenging activity of fruit was determined using the modified ABTS (2, 2, Azinobis, 3 ethyl benzo-thiazolin 6-sulphonic acid) radical depolarization assay. Known amount of aliquot was taken ethanol was added to make up volume to 1 ml. 3 ml of ABTS reagent was added to all the tubes. The discoloration caused reduction of cation by antioxidants from the sample was measured at 734 nm on visible spectrophotometer against alcohol as a blank. Trolox was used as a standard and results are expressed as trolox equivalent [7].

Statistical analysis:

Data were expressed as means ± S.E.M. Statistical analysis was performed by one-way ANOVA. The least significant difference (LSD) test was used for mean comparisons and $P < 0.05$ was considered as statistically significant.

groups	Phenol (mg GAE/100gm)	Flavanoids (mg RE/100 gm)	FRAP (mg TE/100 gm)	DPPH (mg TE/100 gm)	%Inhibition	ABTS (mg TE/100 gm)	% inhibition
Kokum Aqueous	310.98±9.31	298.33±9.46	308.61±2.19	633.06±2.97	33.98±0.16	1005.21±8.68	20.49±0.18
Kokum Methanolic	2414.63±25.39[a]***	4066.66±24.04[a]***	2404.31±10.96[a]***	1515.31±12.08[a]***	40.67±0.32	1556.08±17.35[a]***	15.86±0.18

Results

TABLE: 1 TOTAL ANTIOXIDANT PROPERTIES OF KOKUM

All results depicted in table as Mean ± SEM.

a- comparison between aqueous and methanolic extract of kokum

*: Moderately significance (p>0.05)

**: Significant (p>0.01)

***: Highly significance (p>0.001)

ns: Non significant

TABLE: 2 TOTAL ANTIOXIDANT PROPERTIES OF CINNAMON

All results depicted in table as Mean ± SEM.

groups	Phenol (mg GAE/100gm)	Flavanoids (mg RE/100 gm)	FRAP (mg TE/100 gm)	DPPH (mg TE/100 gm)	%Inhibition	ABTS (mg TE/100 gm)	% inhibition
Cinnamon Aqueous	646.34±18.63	1820±30.55	1349.28±8.77	4577.39±19.19	30.71±0.13	1185.80±6.94	30.21±0.18
Cinnamon Methanolic	3201.22±46.57^{b}***	9000±115.47^{b}***	8411.48±43.85^{b}***	20353.71±114.28^{b}***	54.63±0.31	2226.22±21.95^{b}***	22.69± 0.22

b- comparison between aqueous and methanolic extract of cinnamon

*: Moderately significance (p>0.05)

**: Significant (p>0.01)

***: Highly significance (p>0.001)

ns: Non significant

Figure 1: Phenol (mg TAE/100 gm) content of cinnamon

Figure 2: Phenol (mg TAE/100 gm) content of kokum

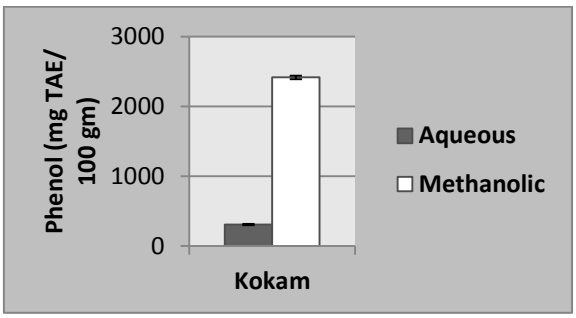


Figure 3: Total flavanoids (mg RE/ 100 gm) content of cinnamon

Figure 7: Total flavanoids (mg RE/ 100 gm) content of kokum

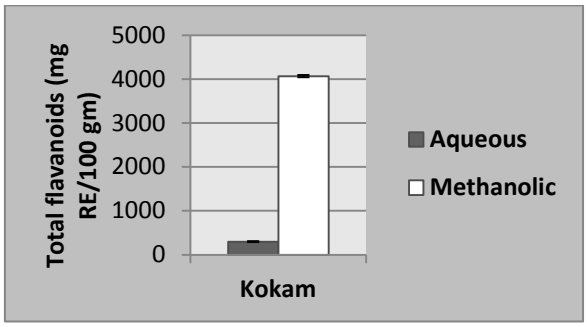


Figure 5: FRAP (mg TE/100 gm) of cinnamon

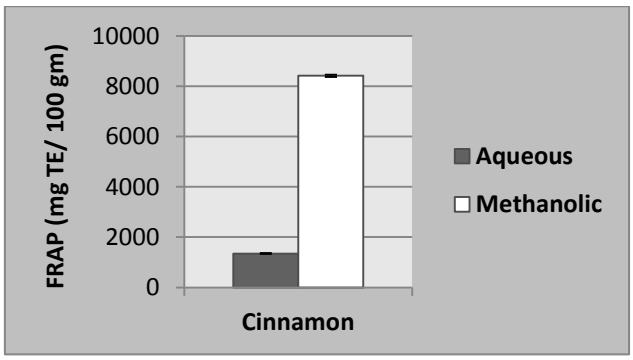


Figure 6: FRAP (mg TE/100 gm) of kokum

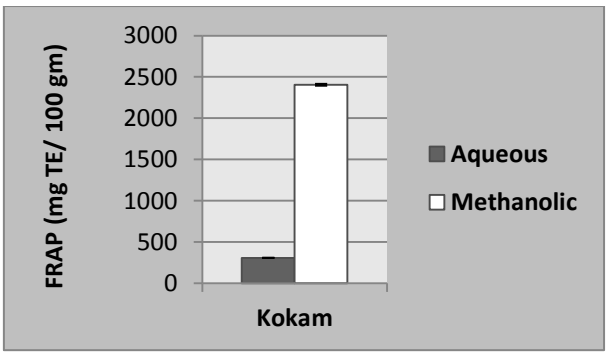


Figure 4: DPPH (% Inhibition) of cinnamon

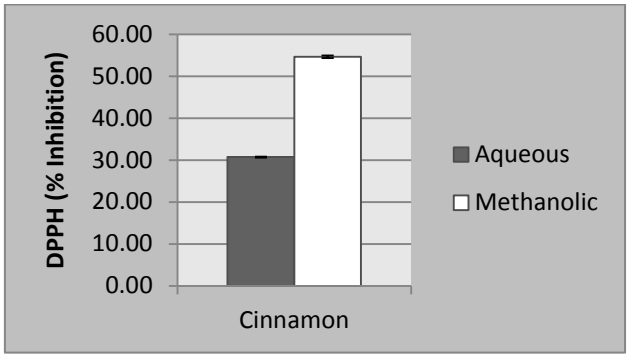


Figure 4: DPPH (% Inhibition) of kokum

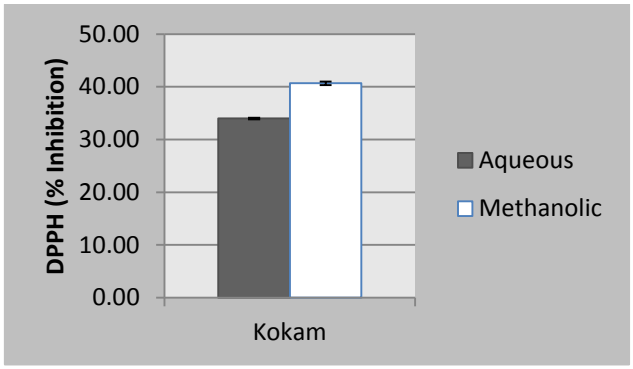


Figure 5: ABTS (% Inhibition) of cinnamon

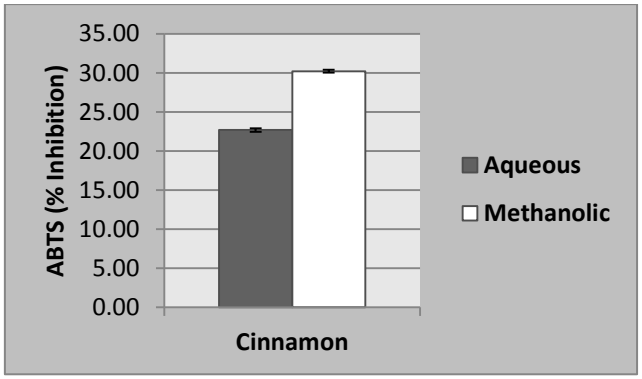
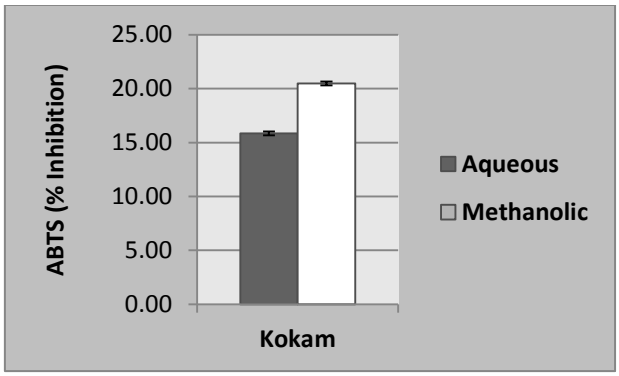


Figure 5: ABTS (% Inhibition) of kokum



Antioxidant Properties of Cinnamon

Cinnamon methanolic extract observed significantly higher compared to aqueous extract (Table- 2). The value of total phenol was 3201.22±46.57 GAE/100 gm, Flavanoid was 9000±115.47 RE/100 gm, FRAP was 8411.48±43.85 TE/100 gm, DPPH was 20353.71±114.28 TE/100 gm and ABTS was 2226.22±21.95 TE/100 gm.

Antioxidant Properties of Kokum

Kokum methanolic extract observed significantly higher compared to aqueous extract (Table- 1). The value of total phenol was 2414.63±25.39 GAE/100 gm, Flavanoid was 4066.66±24.04 RE/100gm, FRAP was 2404.31±10.96 TE/100 gm, DPPH was 1515.31±12.08 TE/100 gm and ABTS was 1556.08±17.35 TE/100 gm.

Discussion

Antioxidant compounds in foods are known to have health beneficial effects. Experimental evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. The potentially imprudent derivatives of oxygen, collectively termed ROS (Reactive oxygen species) such as O_2, H_2O_2 and OH- radical are generated within the body. Overproduction of ROS in the body culminates in oxidative hassle. The ROS induce oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA. This oxidative damage produces a lot of chronic human diseases like diabetes mellitus, cancer, atherosclerosis, arthritis, and neurodegenerative diseases.

Upcoming researches in the area free radical biology supports that incorporation of antioxidants in ones diet could help in combating various diseases. The present study was aimed to evaluate the two most commonly used spice cinnamon and Kokum for their antioxidant properties using aqueous and methanolic extracts. Antioxidant properties measured as total phenol, DPPH, FRAP and ABTS showed that the methanolic extracts had higher antioxidants properties compared to the aqueous extracts. Moreover cinnamon had higher antioxidant index compared to Kokum. Hence incorporation of cinnamon and kokum in ones diet could potentially improve the antioxidant levels. These have been supported by early researches using different animal and human experiments [8].

References

1. Fayaz Pasha, P., & Ramachandran, H. D. (2014). Plant profile, phytochemistry and pharmacology of garcinia indica: a review. World Journal of Pharmacy and Pharmaceutical Sciences, 3(10), 1514-1528.
2. Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y. H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. Journal of food and drug analysis, 22(3), 296-302.
3. Vallverdú-Queralt, A., Regueiro, J., Martínez-Huélamo, M., Alvarenga, J. F. R., Leal, L. N., & Lamuela-Raventos, R. M. (2014). A comprehensive study on the phenolic profile of widely used culinary herbs and spices: rosemary, thyme, oregano, cinnamon, cumin and bay. Food Chemistry, 154, 299-307.
4. Beejmohun, V., Peytavy-Izard, M., Mignon, C., Muscente-Paque, D., Deplanque, X., Ripoll, C., & Chapal, N. (2014). Acute effect of Ceylon cinnamon extract on postprandial glycaemia: alpha-amylase inhibition, starch tolerance test in rats, and randomized crossover clinical trial in healthy volunteers. BMC complementary and alternative medicine, 14(1), 351.
5. Rao, P. V., & Gan, S. H. (2014). Cinnamon: a multifaceted medicinal plant. Evidence-Based Complementary and Alternative Medicine, 2014.
6. Jagtap, P., Bhise, K., & Prakya, V. (2015). A Phytopharmacological Review on Garcinia indica. International Journal of Herbal Medicine, 3(4 Part A), 2-7.
7. Roghelia, V., & Panchal, J. (2016). Antioxidant capacity of cactus pear fruit. World Journal of Pharmaceutical Research, 5(5), 1298-1307.
8. Fayaz Pasha, P., & Ramachandran, H. D. (2015). Plant profile, phytochemistry and pharmacology of garcinia indica: a review. World Journal of Pharmacy and Pharmaceutical Sciences, 3(12), 726-731.

****

Khushbu Dalwadi
Phd Scholar
Department of Home Science
Sardar Patel University
Vallabh Vidyanagar
Anand

Jisha Elias
Research scientist
Medgenome Labs Pvt. Ltd.
Kailash Cancer Hospital and Research Centre
Goraj
Vadodara

Patel V.H
Professor
Professor and Head
P. G. Department of Home Science
Sardar Patel University
Vallabh Vidyanagar

Rema Subhash
Professor
Department of Home Science
Sardar Patel University
Vallabh Vidyanagar
Anand

Copyright © 2012- 2016 KCG. All Rights Reserved. | Powered By: Knowledge Consortium of Gujarat