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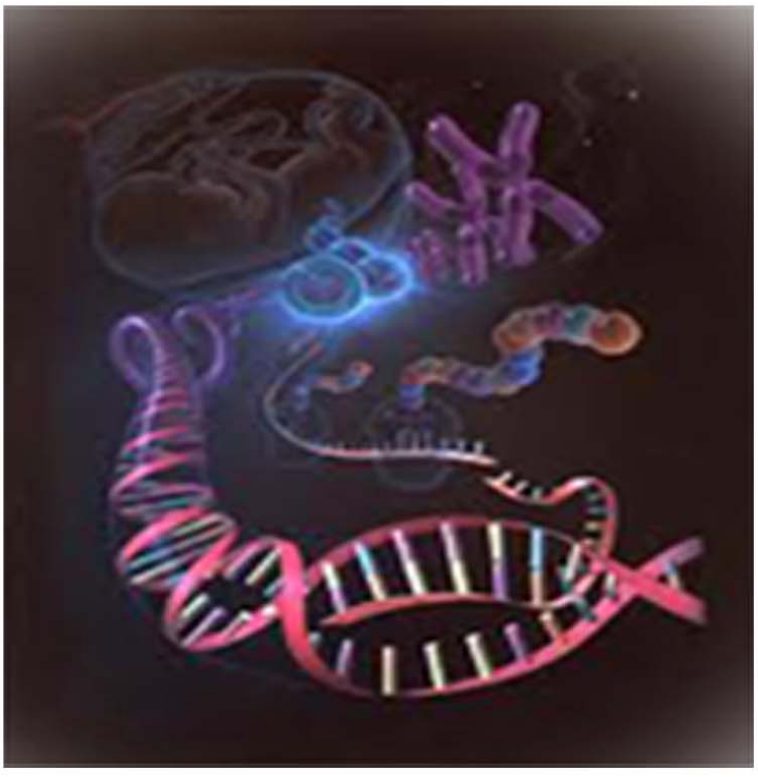

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*Research Paper*

# A STUDY ON ANTIOXIDANT ACTIVITY OF SOME COMMONLY USED SPICES IN INDIA

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Antioxidants are vital molecules which protect the body from harmful effects caused by free radical induced oxidative stress. Proper diet is the best source of free radical scavenging antioxidants to the body of any organism. The antioxidant activity of spices that are used in India has not been studied well. Indians use a variety of spices in their cooking recipes. The main objective of this study is to assess the antioxidant activity of some commonly used spices by Indians such as Fenugreek seeds (*Trigonella foenum*), Mustard seeds (*Brassica nigra*), Black pepper (*Piper nigram*), Poppy seeds (*Papaver somniferum*), Coriander seeds (*Coriandrum sativum*), Cinnamon bark (*Cinnamoum* sps), and Cumin seeds (*Cuminum cyminum*). The hydroxyl radical is an extremely reactive species which reacts at a high rate with all types of molecules in the body like proteins, lipids, nucleic acids, sugars and create oxidative stress, which plays a major role in the development of chronic and degenerative disorders such as Cancer, Auto-immune disorders, Rheumatid arthritis, Cataract, Aging, Cardiovascular and Neuro degenerative diseases. The present study revealed that the scavenging activity of spices that are examined ranged from 18.84 µg/ml to 222.90 µg/ml. Among the selected spices *Papaver somniferum* seeds shown the maximum scavenging activity, i.e., 224.54 ± 17.27 µg/ml, where as *Cinnamoum* species bark showed minimum scavenging activity, i.e., 18.18 ± 10.91 µg/ml. The present study revealed that the use of spices in the cooking has good advantage and hence, it is preferred to use spices in daily diet, as this will have good hydroxyl radical scavenging activity which protects from chronic and degenerative ailments due to oxidative stress.

***Keywords:*** Antioxidant activity, Hydroxyl radical scavenging activity, Oxidative stress, Spices

## INTRODUCTION

Consumers are demanding natural foods that show antioxidant property which enhance health and food preservation and hence developed a great demand for antioxidants derived from natural resources. In response there increased a greater demand for natural dietary products which produce antioxidants in the body (Barlow, 1990 and Rice-Evans *et al.,* 1997).

Antioxidants are the compounds which inhibit

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the oxidation reactions caused by free radicals. Free radicals which are also called as Reactive Oxygen Species (ROS) or Active Oxygen Species (AOS), are produced during various metabolic cellular processes. ROS include free radicals such as hydroxyl radicals ($OH^-$), superoxide ions ($O_2^-$), nitric oxide ($NO^-$), alkyl oxide ($RO^-$), alkyl carboxylic acid ($ROO^-$) and non-free radicals such as hydrogen peroxide ($H_2O_2$), singlet oxygen ($O^-$), hypo chloride radicals ($HClO^-$) (Halliwell, 1995) and Odukoya *et al.*, 2005). Normal range of production of ROS helps in the regulation of cell proliferation, intercellular signalization, phagocytosis and also synthesis of biologically active compounds. But hyper production of ROS develops oxidative stress which causes cardiovascular diseases, diabetes, tumors, rheumatoid arthritis, epilepsy, mutagenesis, carcinogenesis, arteriosclerosis, Alzheimer's disease, tissue injury (Halliwell and Gutteridge, 1989; Alho and Leinonen, 1999).

Spices are the best sources of polyphenolic compounds such as flavoids, flavanoids, phenolic compounds, anthocyanins, phenylpropanoids, anthraquinones which are good antioxidants (Graham, 1992; Rice-Evans *et al.,* 1996). Piper species, commonly used in diet and traditional medicine, were assessed for their antioxidant potential (Karthikeyan and Rani, 2003). The antioxidant and radical scavenging activities of Black pepper seeds have been well reported for both water and ethanol extracts exhibited strong antioxidant activity, anti-microbial, larvicidal and anti-cancer activities (Gulcin, 2005 and Park *et al.,* 2002). A large number of medicinal plants and their purified compounds have shown beneficial therapeutic potentials (Aqil *et al*., 2006).

Hence the present study has been aimed to study the comparative studies of the antioxidant activities of Fenugreek seeds (*Trigonella foenum-graceum*), Mustard seeds (*Brassica nigra*), Black pepper (*Piper nigrum*), Poppy seeds (*Papaver somniferum*), Corainder seeds (*Coriandrum sativum*), Cinnamon bark (*Cinnamomum* sps) and Cumin seeds (*Cuminum cyminum*).

# MATERIALS AND METHODS

## Materials

The dried selected plant materials, Fenugreek seeds (*Trigonella foenum-graceum*), Mustard seeds (*Brassica nigra*), Black pepper (*Piper nigrum*), Poppy seeds (*Papaver somniferum*), Corainder seeds (*Coriandrum sativum*), Cinnamon bark (*Cinnamomum* sps) and Cumin seeds ( *Cuminum cyminum*) were obtained at the Rythu Bazar Municipal Market in Hanamkonda city, Warangal, Andhra Pradesh, India. The radicals and all the solvents used were of analytical grade.

## Preparation of the Extracts

Before extraction, all the dried spices collected were ground in an electric grinder into fine powder. 50 g of each sample were extracted with 250 ml methanol on a table shaker at 120 rpm for 24 h. Then the extract was filtered, and the residue was re-extracted with 50 ml methanol for 3 h. The filtrates were allowed to evaporate the complete methanol in the Petri dishes and then 10 ml methanol is added to each Petri dish and centrifugation done at 3000 rpm for 20 min. The crude extracts were diluted using methanol according to the assay needs.

## Determination of Antioxidant Activity by Hydroxyl Radical Scavenging Assay

Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity. This

method involves *in-vitro* generation of hydroxyl radicals using $Fe^{3+}$/Ascorbate/EDTA/$H_2O_2$ system using Fenton reaction (Klein *et al.,* 1968; Klein and Henning, 1969). Scavenging of this hydroxyl radical in presence of antioxidant is measured. In one of the methods the hydroxyl radicals formed by the oxidation is made to react with DMSO (Dimethyl sulphoxide) to yield formaldehyde. Formaldehyde formed produces intense yellow color with Nash reagent (2 M Ammonium acetate with 0.05 M Acetic acid and 0.02 M Acetyl acetone in distilled water). The intensity of yellow color formed is measured at 412 nm spectrophotometrically against reagent blank. The activity is expressed as % hydroxyl radical scavenging (Babu *et al.,* 2001).

A volume of 1 ml of a known dilution extracts (10 mg/ml of the methanol extract) were taken separately and 1 ml of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of EDTA (0.018%) and 1ml of DMSO (0.85% v/v in 0.1 M phosphate buffer, pH 7.4) were added to each extract. The reaction was initiated by adding 0.5ml of 0.22% Ascorbic acid. Test tubes were capped tightly and heated on a water bath at 80°C-90°C for 15 min. The reaction was terminated by addition of 1 ml of ice-cold TCA (17.5% w/v). 3 ml of Nash reagent (75 g of ammonium acetate, 3 ml of glacial acetic acid and 2 ml of acetyl acetone were mixed and made up to 1 L with distilled water) was added to all of the test tubes and left at room temperature for 15 min for color development. The intensity of the yellow color formed was measured spectrophotometrically at 412 nm against reagent black (Thabrew *et al.,* 1998). The experiment was repeated in triplets and L-Ascorbic acid was used as the standard. The hydroxyl radical scavenging activity of the extract is reported as % inhibition of hydroxyl radical generation and calculated as:

$$\text{OH-Scavenged (\%)} = \frac{(A_{con})-(A_{test})}{(A_{con})} \times 100$$

where

$A_{con}$ is the absorbance of the control (L-Ascorbic acid) reaction; and

$A_{test}$ is the absorbance in the presence of the sample of the extracts (Yerra Rajeshwar *et al.,* 2005).

## RESULTS AND DISCUSSION

Natural antioxidants that are present in spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress (Lee *et al.,* 2004). Spices contain free radical scavengers like polyphenols, flavonoids and phenolic compounds. In the present paper, we have evaluated the hydroxyl radical scavenger activity of methanolic extracts of Fenugreek seeds (*Trigonella foenum-graceum*), Mustard seeds (*Brassica nigra*), Black pepper (*Piper nigrum*), Poppy seeds (*Papaver somniferum*), Corainder seeds (*Coriandrum sativum*), Cinnamon bark

**Table 1: *In Vitro* Antioxidant Activity of Methanolic Extract of Selected Spices**

| Test Compound (Methanolic Compound) | % inhibition of Hydroxyl Radical Generation per mg/ml |
|---|---|
| Control(L-Ascorbic acid) | 24.34±0.27 |
| *Trigonella foenum-graceum* | 66.66±0.29 |
| *Brassica nigra* | 42.10±0.5 |
| *Piper nigrum* | 128.07±1.41 |
| *Papaver somniferum* | 224.56±17.27 |
| *Coriandrum sativum* | 70.17±0.32 |
| *Cinnamomum sps* | 18.18±10.91 |
| *Cuminum cyminum* | 159.64±2.25 |

(*Cinnamomum* sps) and Cumin seeds (*Cuminum cyminum*).

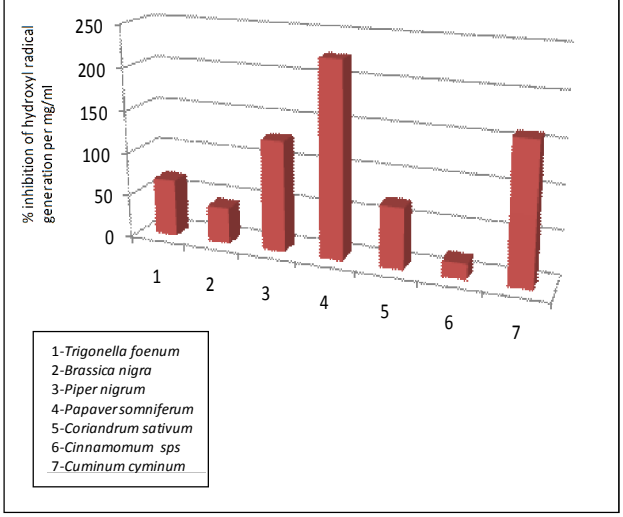
Among the seven extracts and standard tested for the *in vitro* antioxidant activity are shown in Table 1 using Hydroxyl Radical Scavenging Assay method, the crude methanolic extracts of *Papaver somniferum* seeds shown the maximum scavenging activity, i.e., 224.54 ± 17.27 µg/ml, where as *Cinnamomum* species bark showed minimum scavenging activity, i.e., 18.18 ± 10.91 µg/ml. The results indicate that the antioxidant activity of the crude extract of *Cinnamomum* sps showed lower than that of ascorbic acid. The all other selected spices for the study except *Cinnamomum* sps showed higher antioxidant activity than that of ascorbic acid .The ranking in terms of antioxidant capacity as measured by Hydroxyl Radical Scavenging assay followed the following decreasing order:

*Papaver somniferum> Cuminum cyminum> Piper nigrum> Coriandrum sativum> Trigonella foenum-graceum> Brassica nigra> Cinnamomum* sps.

But when we compare to the Nigerian or Thai or Arab spices to Indian spices, Nigerian spices of Pepper seeds shown IC 50 value of 76.94±2.64 µg/ml (Odukoya *et al.*, 2005). Arab spices of pepper seeds shown IC 50 value of 144.1±2.2 µg/ml, where as in the present study revealed that Indian spices of pepper seeds shown 128.07±1.41 µg/ml. Nigerian spices shown higher antioxidant activity when compared to Indian spices to Arab spices.

Arab spices of fenugreek seeds shown IC 50 value of 444.1±5.5 µg/ml (Nooman A Khalaf, 2008), where as Indian spices of fenugreek seeds in the present study shown 66.66±0.29 µg/ml. When compared between Indian and Arab spices of fenugreek seeds, Indian spices shown more antioxidant activity than Arab spices.

**Figure 1: Graph Showing Antioxidant Activity of Methanolic Extracts of Selected Spices**




In these results we have focused on methanolic extracts obtained from 7 spices commonly used in regular diet for their taste and flavor, which have rarely been reviewed. Results of these antioxidant activities of selected spices using hydroxyl radical scavenging activity were presented. This analysis technique could provide help into the variations in the antioxidant profiles between different spices and could help disease prevention and cure using simple herbs and spices. Spices can also potentially substitute the synthetic antioxidants in foods to prevent oxidative deterioration. So eating spices in daily regular diet is good as it keeps us away from many diseases naturally.

When we compare to the all spices used in the world it is found that eating spices along with daily diet will improve the health as they act as

good antioxidant activity and help us to escape us to become victims of many diseases

## CONCLUSION

The tested plants were found to be rich in antioxidant sources. Hence they are recommended as good food supplement in daily diet which act as natural antioxidants that can protect us from many diseases caused by ROS. Further research is needed on the determination of the correlation between the antioxidant capacity and the chemical composition of the bioactive compounds in the plant extracts.

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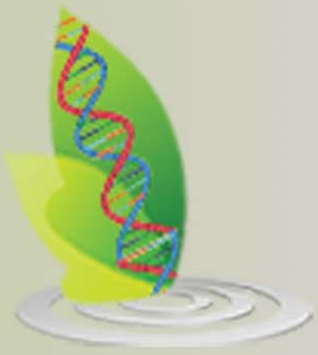

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