

**EVALUATION OF PHYTOCHEMICAL AND ANTIOXIDANT
COMPOUNDS OF SOME INDIAN SPICES****Prasad. M. P***

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Article Received on
07 October 2014,

Revised on 29 Oct 2014,
Accepted on 20 Nov 2014

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ABSTRACT

Many of the spices and herbs used in day to day cooking have been valued for their medicinal properties in addition to their flavour and fragrance qualities. They have been used in traditional medicines for ages. Herbs and spices have been extensively used as food additives and as natural antioxidants. The present study aims at evaluating the phytochemical compounds and antioxidant properties of spices which are used in south Indian cooking. A total of 10 spices were used in the present investigation namely Methi, Cloves, Cumin, Pepper, Bay leaf, Cinnamon, Saunf, Cardamom, Ajwain, and Mustard seeds. The phytochemical screening and quantitative estimation of the crude yields studied showed that the samples were rich in alkaloids, flavonoids, tannins and saponins. Bay leaf extract showed highest amount of tannic acid. The cloves sample extracted with methanolic

Solvent showed a protein content of 7.0% followed by cardamom and saunf with a protein content of 5.5%. The DPPH assay of alcoholic extract of carried out and ajwain showed the lowest value Of 300µg, which indicated that the concentration is able to reduce the DPPH to 50% from its initial concentration. Mustard, cloves, saunf and cumin showed DPPH reduction above 1mg concentration.

KEYWORDS: Antioxidant, DPPH, Medicinal Plants, Spices.

INTRODUCTION

Medicinal plants play a fundamental role in traditional medicine. Plants are rich in a wide variety of secondary metabolites such as Cardiac glycosides, Tannins, Terpenoids, Phenols, Alkaloids and flavonoids which have been found to have medicinal properties. Antibacterial

compounds of various plant parts like root, stem, leaves, seeds, flowers have been well documented for some of the medicinal plants for past two decades. ^[1] Plant secondary metabolites are found to be an excellent source of Phenolic phytochemical, especially as an antioxidant and antimicrobial compound. Crude extracts of some well known medicinal plants are used to control plant pathogens. The use of plant extracts and phytochemicals, both with known antimicrobial properties, are of great significance to therapeutic treatments ^[2]. The antioxidant property of any plant material is due to the presence of many active photochemical compounds including Vitamins, Flavonoids, Terpinoids, Carotenoids, Coumarins, Curcumins, Lignins, Saponin and plant Steroids. ^[3, 4, 5, 6]

Herbs and Spices are common ingredients in the Indian cuisine which enhance the taste and flavour of the food substances. Herbs and spices have been extensively used for its medicinal properties in the Indian folklore and even today as homemade remedies in the rural India and also as food additives for natural antioxidants. Besides flavouring, spices and herbs have for ages been used for their medical and antiseptic properties. The preservative effect of many spices and herbs suggest the presence of antioxidative and antimicrobial constituents. Spices and aromatic herbs are considered to be essential in diets or medical therapies for delaying ageing and biological tissue deterioration. ^[7] Studies have shown that Fenugreek leaves and seeds are rich in Proteins, Carbohydrates, Flavonoids, Aminoacids, and Saponins. ^[8] Fenugreek aids in problems related to stomach indigestion and also may aid with insulin secretion, as suggested by animal studies, since typically these patients have little or no endogenous insulin production. ^[9]

Piper nigrum Linn. is a flowering vine in the family Piperaceae which is commonly used as a spice in almost all food and diet and in traditional medicines. The antioxidant and radical scavenging activities of black pepper seeds have been previously reported by Gulcin ^[10]. The methanolic extract of *Piper* spp; has exhibited strong antioxidant and antimicrobial activity, larvicidal activity ^[11]. It is also found to be effective against patients suffering from asthma. Clove (*Eugenia caryophyllus*) belonging to family Myrtaceae is a commonly used household spice. Antioxidant, Superoxide dismutase and Catalase activities have been reported. ^[12, 13, 14]

MATERIAL AND METHODS

Collection, processing and extraction of plants: Methi, Cloves, Cumin, Pepper, Bay leaf, Cinnamon, Saunf, Cardamom, Ajwain, and Mustard seeds samples were collected from local super markets in Bangalore. The samples were washed with tap water shade dried for around

a week and were powdered using a grinder. The samples (5Grams of powder) were taken in 50 ml of methanol and were extracted on a rotary shaker for 48hrs. The above methanol extract was filtered and further used for phytochemical screening, quantitative analysis, antioxidant activity.

Phytochemical screening

Qualitative Analysis

Test for Reducing sugars (Fehlings test): 0.5 ml of the methanol extract (sample) was taken in a test tube and 0.5 ml of Fehling's A solution and 0.5 ml of Fehling's B solution was taken in the above test tube. The test tube was kept for boiling in water bath. The solution was observed for colour reaction. Positive test gives a brick red colour.

Test for Cardiac glycosides (Keller Killiani test)

To 0.5 ml of the methanol extract diluted to 5 ml in distilled water and 2 ml of glacial acetic acid and one drop of 1% ferric chloride solution was added. This was under layered with 1 ml of concentrated sulphuric acid. A Brown ring at the interface indicated the presence of a deoxy sugar characteristic of cardenolides. A violet ring appear's below the brown ring; while in acetic acid layer is greenish ring may form just above the brown ring and gradually spread throughout this layer.

Test for Anthraquinones

0.5 ml of the methanol extract was boiled with 3 ml of sulphuric acid and filtered while hot. The filtrate was shaken with 1.5 ml of chloroform. The aqueous layer was pipetted into another test tube and 1ml of dilute ammonia was added. The resulting solution was observed for colour changes.

Test for Terpenoids (Salkowski test)

To 0.5 ml of the methanol extract was added to 2 ml of chloroform. Concentrated sulphuric acid 3 ml was carefully added to form a layer. Positive test gives a reddish brown colouration of the interface indicates the presence of terpenoids.

Test for Proteins (Lowry's method)

To 0.5 ml of methanolic extract of samples 2.25 ml of reagent C each and 0.25 Folin cio-calceateau reagent was added. The test tube containing the above reaction mixture was incubated at room temperature for 20 minutes. Positive test gives an indication of violet colour.

Test for Flavonoids

Test 1: 5ml of dilute ammonia was added to a portion of aqueous filtrate of the methanolic extract. Concentrated sulphuric acid 1ml was added. A yellow colouration that disappears on standing indicates the presence of flavonoids.

Test 2: A portion of methanolic extract of samples of sample was heated with 10 ml of ethyl acetate over a steam bath for 3 minutes. The mixture was filtered and 4 ml of filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration indicates the presence of flavonoid.

Test for Saponins

To 0.5 ml of methanolic extract of samples, 5 ml of distilled water was added to a test tube. The test tube was heated to a boil and was observed for emulsion formation.

Test for Tannins

About 0.5 ml of the extract was boiled in 10 ml of water in a test tube and then filtered. Few drops of 0.1 % ferric chloride were added. Positive test gives a brownish green or a blue-black coloration.

Test for Lignins (Maule's test)

To 0.5 ml of extract, 1% of potassium permanganate solution and few drops of ammonium chloride solution and a drop of ammonium hydroxide was added and observed for colour changes. Red rose colour is the indicator of the presence of lignins.

Test for Phenols

A portion of methanolic extract of samples was treated with neutral ferric chloride solution and observed for the result. Positive test gives Greenish black or yellow colours.

Test for Alkaloids

To 0.2 ml of methanolic extract of samples, 2% sulphuric acid was added warmed for 2 minutes. Filtered and few drops of Dragendorff's reagent was added and observed for colour change. Positive test gives an Orange red precipitate.

Quantitative analysis

Estimation of Tannins: Different aliquots of 25-1000 µl of tannic acid working standard of concentration range of 0.25-10 µg were pipetted into different test tubes. Volume was made

up with 1ml distilled water. All the above test samples were made up with 2.5 ml of sodium bicarbonate. Blank was prepared by mixing 1ml of distilled water and 2.5 ml of 10% sodium bicarbonate. The test tubes were incubated in room temperature for 40 minutes. O.D was read at 725 nm for the above standard and samples. A graph of optical density versus concentration of tannic acid ($\mu\text{g/ml}$) was plotted and the unknown concentration of samples was determined from the above standard graph.

Estimation of Phenols

Different aliquots of 25-1000 μl of Gallic acid working standard of concentration range of 0.25-10 μg were pipetted into different test tubes. Volume was made up with 1ml distilled water. All the above test samples were made up with 1.2 ml of sodium carbonate and 1.5 ml of FC reagent. Blank was prepared by mixing 1ml of distilled water and 1.2 ml of sodium carbonate and 1.5 ml of FC reagent. The test tubes were incubated in room temperature for 30 minutes. O.D was read at 765 nm for the above standard and samples. A graph of optical density versus concentration of Gallic acid ($\mu\text{g/ml}$) was plotted and the unknown concentration of samples was determined from the above standard graph.

Estimation of Reducing sugars

Different aliquots of 25-1000 μl of glucose working standard of concentration range of 0.25-10 μg were pipetted into different test tubes. Volume was made up with 1ml distilled water. All the above test tubes were made up with 3 ml of DNS solution. Blank was prepared by mixing 1ml of distilled water and 3 ml of DNS solution. The test tubes were incubated in water bath at 100°C for 15 minutes. 1 ml of rochette salt solution was added to all test tubes. O.D was read at 575 nm for the above standard and standard. A graph of optical density versus concentration of glucose ($\mu\text{g/ml}$) was plotted and the unknown concentration of samples was determined from the above standard graph.

Estimation of Flavonoids

1 gm of plant sample was extracted with 10 ml of 80% methanol at room temperature for 2 hours and Centrifuged at 5000 rpm for 6 minutes. Solution was filtered through whatmann filter paper number 1. The filtrate was transferred into a crucible, evaporated till dryness over a water bath, weighed to a constant weight. The percentage of flavonoid was calculated.

$$\text{Flavonoid content (\%)} = \frac{\text{weight of flavonoid (gm)}}{\text{weight of sample (gm)}} \times 100$$

Antioxidant activity

DPPH scavenging activity: To determine the antioxidant activity of plant extracts and natural compounds, DPPH is the standard procedure for radical scavenging assay. The free radical scavenging activity of methanolic leaf extract was measured by using the method described by Dehshahri ^[15], with slight modification.

100µl extract were mixed with 1ml of methanolic solution of 0.1mM DPPH. Mixture was shaken well and incubated at room temperature for 30 minutes. And the absorbance was measured at 517nm in a UV spectrophotometer.

Percentage inhibition was calculated from control using the equation.

$$\text{DPPH scavenging effect (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Reducing power method

The reducing power was determined according to the method of Isabel Ferreira et al (2005). Various plant samples of 0.5 ml were taken. To this, 2.5ml of 0.2M phosphate buffer (pH-6.6) and 2.5ml of 1% potassium ferricyanide was added. The mixture was incubated at 50°C for 20minutes. To this 2.5ml of 10% Trichloro-acetic-acid was added. The mixture was centrifuged at 3000 rpm for 10 minutes. The upper layer was mixed with 2.5ml of distilled water. To this 2.5ml of 0.1% of ferric chloride was added, and the absorbance was read at 700 nm.

RESULTS

Samples



Cumin



Methi



Cardamom



Cinnamon



Saunf

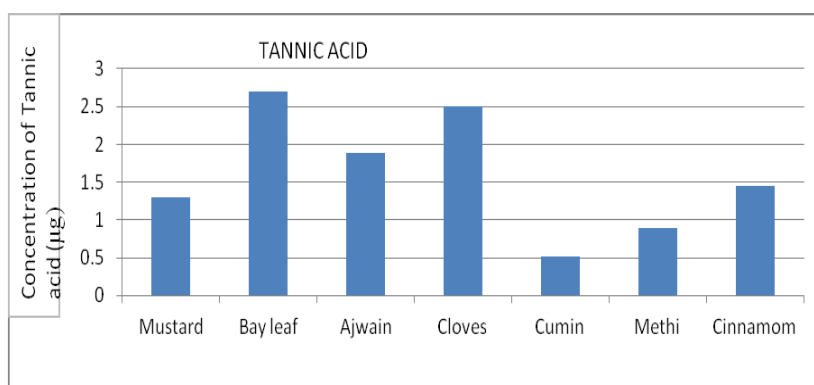


Mustard

**Bay leaf****Ajwain****Pepper****Cloves**

Qualitative analysis: The qualitative assay of ten medicinal spices samples were screened as described in the methodology for their phytochemical constituents. The phytochemical screening and quantitative estimation of the crude yields studied showed that the samples were rich in alkaloids, flavonoids, tannins and saponins.

The result obtained by the methanolic extract possessed all the active constituents except anthraquinones table 1.

**Figure 1: Qualitative analysis of Tannic acid**

Quantitative analysis: The quantitative assay for tannins, reducing sugars, phenols and flavonoids were followed by Sofowara ^[16], Trease and Evans ^[17] and Harborne ^[18]. The results obtained are showed in Figure 1-4.

The proteins, tannins and reducing sugars are the primary metabolites of plants, necessary for cellular processes while phenol and flavonoid are the secondary metabolic compounds produced in response to stress, such as the case when acting as a deterrent against herbivores as mentioned by Kim ^[19].

Table 1: Qualitative analysis

Botaical Name	Commo n name	Reducin g sugar	cardiac glycosides	Terpen oids	Sapon ins	Tan nins	Phe nol	Prot eins	Anthra- quinones	Flavo noid	Alkaloids
Brassica nigra	Mustard	+	+	+	+	+	++	+	-	+	+
Laurus nobilis	Bay leaf	-	++	++	-	+	+	+	-	+	-
Trachyspermum copticum	Ajwain	+	++	++	-	++	++	+	-	+	+
Elettaria cardamomum	Cardamom	+	+	++	-	-	-	+	-	+	-
piper nigrum	Pepper	+	++	++	-	-	-	+	-	+	+
Syzygium aromaticum	Cloves	+	+	++	+	++	++	+	-	+	+
Cuminum cyminum	Cumin	+	++	++	-	++	++	+	-	+	-
Trigonella foenum graecum	Methi	+	+	++	+	+	+	+	-	++	+
Cinnamomum verum	Cinnamon	+	++	++	-	++	+	+	-	+	-
Foeniculum vulgare	Saunf	+	++	++	-	-	+	+	-	+	-

Quantitative analysis

The quantitative assay for tannins, reducing sugars, phenols and flavonoids were followed by Sofowara ^[16], Trease and Evans ^[17] and Harborne ^[18]. The results obtained are showed in Figure 1-4.

The proteins , tannins and reducing sugars are the primary metabolites of plants , necessary for cellular processes while phenol and flavonoid are the secondary metabolic compounds produced in response to stress , such as the case when acting as a deterrent against herbivores as mentioned by Kim ^[19].

The methanolic extract of the samples were checked for the qualitative analysis of tannic acid and Bay leaf extract showed highest amount of tannic acid when compared to the standard tannic acid concentration.

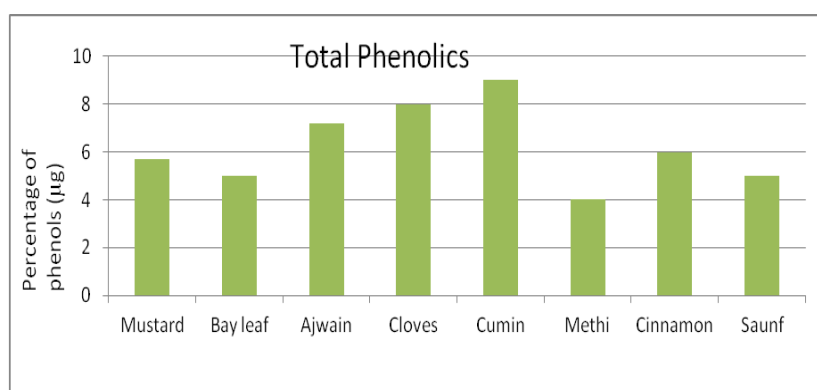


Figure 2: Qualitative analysis of Total phenols

The total phenolic content plays an important role in the composition of the samples. The methanolic extract of cumin showed greater phenolic content and methi extract showed only 4.0% of the phenolic content.

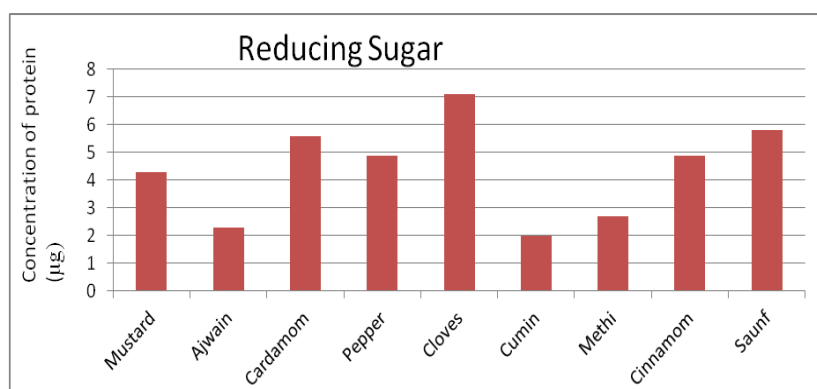


Figure 3: Qualitative analysis of Reducing sugar

Reducing sugar assay is carried out to determine the concentration of proteins present in the samples. The cloves sample extracted with methanolic solvent showed a protein content of 7.0% followed by cardamom and saunf with a protein content of 5.5%.

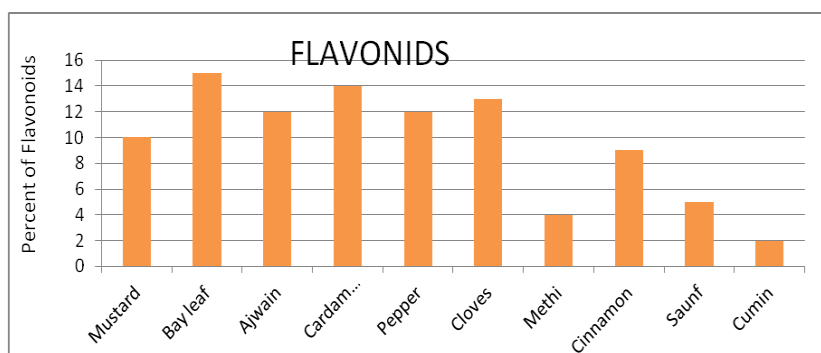


Figure 4: Qualitative analysis of Flavonids

Flavonoids are one of the important constituent present in most of the plant samples. The estimation of flavonoids was carried out and was determined that most of the sea weeds samples have a high amount of flavonoids, the maximum flavonoid was seen in bay leaf sample when extracted with methanolic solvent.

Antioxidant Assay

DPPH

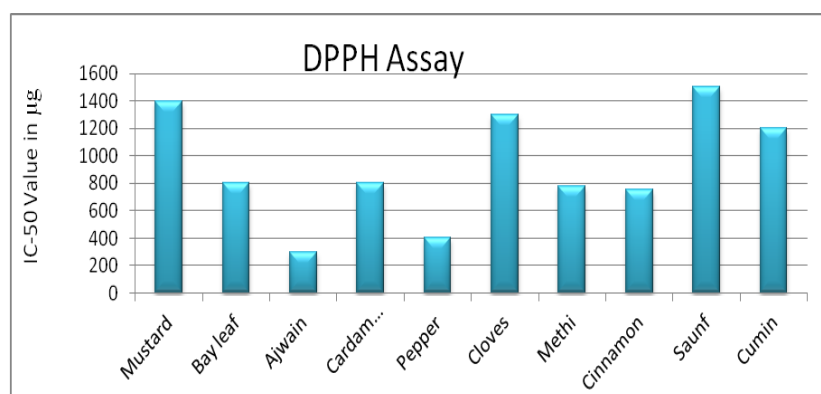
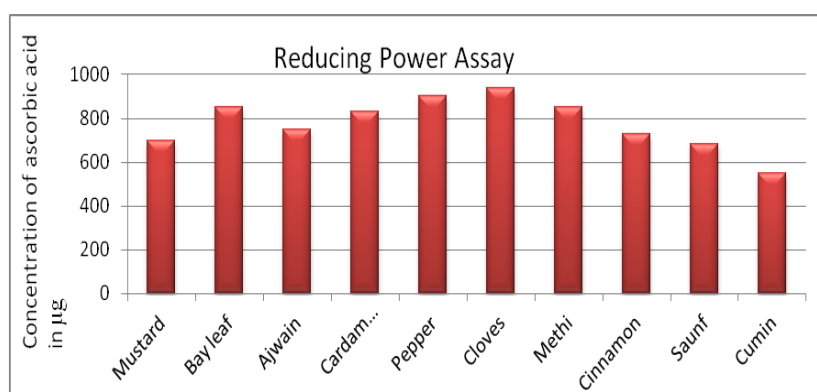


Figure 5: DPPH Assay- IC50 value.

The sea weed were checked for their antioxidant property by the methanolic solvent extracts and was determined that all the samples showed antioxidant property. The DPPH reduced with the increase in the concentration of the samples. The alcoholic extract of ajwain showed the lowest value Of 300µg, which indicated that the concentration is able to reduce the DPPH to 50% from its initial concentration. Mustard, cloves, saunf and cumin showed DPPH reduction above 1mg concentration (Figure 5).

Reducing power



Reducing power assay for the crude alcoholic extract was carried out to determine the ascorbic acid concentration. All the samples showed the presence of ascorbic acid concentration. The concentration varied from a range of 500-1000 µg. The cloves sample showed ascorbic acid concentration of 900 µg, the cumin extract showed the lowest ascorbic acid concentration of 540 µg (Figure 6).

DISCUSSION

Aneta Wojdyło et al., 2007^[20] investigated Total equivalent antioxidant capacities (TEAC) and phenolic contents of 32 spices extracts from 21 botanical families grown in Poland and found that Many spices had high levels of phenolics and exhibited high antioxidant capacity.

M. Badrul Alam et al., 2011^[21], studied the antioxidant property of *Brassica nigra* and showed that IC 50 value was 63.09 µg/ml whereas the standard antioxidant showed IC 50 value 14.45 µg/ml in DPPH method.

Iris Hinneburg et al., 2006^[22], carried out the investigation for determining the total phenolic content and antioxidant properties from extracts from basil, laurel, parsley, juniper, aniseed, fennel, cumin, cardamom, and ginger. The extracts from basil and laurel possessed the highest antioxidant activities except for iron chelation. Although parsley showed the best performance in the iron chelation assay, it was less effective at retarding the oxidation of linoleic acid. In the linoleic acid peroxidation assay, 1 g of the basil and laurel extracts were as effective as 177 and 212 mg of trolox respectively. Thus, both extracts are promising alternatives to synthetic substances as food ingredients with antioxidant activity.

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