Extraction of Antioxidants from spices

Received for publication, April 20, 2020, and in revised form, May 5, 2020

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Abstract

Spices are an important part of our diet mainly because of their taste. They add a great taste to the prepared food. Since the ancient days spices and herbs are also known for their medicinal properties because they are used for treating various kind of illness like cough and cold, diabetes, common cold, gastric ulcer, different types of skin diseases and so on. In the present days, spices are available in different forms and different kinds of non permitted dyes are added to it mainly to protect its texture. These non permitted dyes are harmful and can cause serious illness if consumed regularly.The aim of this project is to estimate, extract and characterize the antioxidants and bioactive molecules from spices. Not only this, the presence of artificial colours are also tested in the spices. Six different spices were taken that we use in our daily life and they are also available easily in the market. They are taken as three different forms (the organic form, the whole spice and the powdered form available in the market) for the experiment.All the spices were treated with aqueous ethanol and polyphenols were estimated with the help of Folin Ciocalteau reagent. The absorbance are recorded using spectrophotometer. The presence of dye like sudan and rhodamine are tested with the help of ethyl acetate and methanol respectively. The functional group are also identified with the IR spectroscopy.

Keywords: Spices, Medicinal propertires, Folin CIocalteau reagent, Artificial colours

Introduction

An antioxidant can be defined as a molecule capable of inhibiting the oxidation of other molecules. Oxidation produces free radicals which can then start chain reactions which ultimately lead to damaging of cells. Antioxidants prevent these chain reactions by removing the free radical intermediates and also inhibit other oxidation reactions. Antioxidants are sometimes called “free-scavengers.” The source of antioxidants can be natural such as caretonoids or artificial such as BHT. The antioxidants are also produced in the body and they are known as endogenous antioxidants and the antioxidants that come from outside the body and they are known as exogenous antioxidant. Antioxidants can protect lipids and oils in food from oxidative

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degradation. When antioxidants are added to food they control the rancidity development, retard the formation of toxic substances. Antioxidants are often added to food to maintain the nutritional quality and to increase the shelf life. Natural antioxidants can be obtained from edible products such as spices and herbs, which are easily available. Oxidative stress, which is caused by the high concentration of free radicals can be reduced by natural antioxidants [1]. Natural antioxidants can be induced by various factors like UV rays, gamma rays, X-ray radiation, polluted food, psycho emotional stress, smoking, intensive physical extension, adverse environmental condition, alcoholism, drug addiction and so on. Oxidative stress is often linked to heart disease, cancer, stroke, arthritis, respiratory problems, immune deficiency. Parkinson's disease and so on[2].

A spice may be defined as a dried part of the plant which may be a root, leaf, bark which is primarily used for flavoring, colouring or preserving food substances. Not only these, spices also have other properties like antimicrobial properties. Spices can also be used in the manufacture of medicine, cosmetics, perfumes and so on. Spices are an important diet constituent all across the world. They are mainly used because of their flavour, but since the ancient times, spices and herbs are also known for their medicinal properties. Almost 80% of the world's population mainly depends exclusively on the plants and herbs for their medicinal properties for healing, where as in the developed world, preference Synthetic food colours are being used widely in food as well as in spices mainly in the powdered form to avoid the loss of original color and also to make the product attractive to the customer. Some food colours are permitted for use while some are not used. Some food colours may even cause serious illness. Some food colours that are permitted under the provision of Food Adulteration Act(1945), which includesis given more to the pharmaceutical medicine and surgery.

Three Red shades- Carmoisine, ponceau 4R, Erythrosine
Two yellow shades- Sunset yellow-FCF and Tartrazine
Two blue shades- Brilliant blue FCF, carmine
Green shade- Fast green FCF

But other than these permitted dyes some unpermitted dyes such as rhodamine, sudan III, IV, metanil yellow are commonly used in food industeies and scientific research. Scientific research include staining of lipid with sudan IV for visualization and analytical purpose. Sudan IV is commonly used as a food adulterant in red chilli powder and foods haivng red chilli like curry powder, meat masala, frozen mix and spice mixes. It is commonly used as it is cheaper and it gives an intense orange-red colour. Not only these, sudan dyes are also used in cosmetic products and animal testing and it is found that sudan III causes allergic reaction (International Agency for Research on cancer, 1975). These dyes can be an origin of allergic reaction, eczema, skin dermatoses (Jaskot and Costa, 1994) which affects liver, lungs and the vasculatory system, immune system and the reproductive system.

Another dye which is commonly used in food processing industries is rhodamine. It is banned by the Government of India as per PFA act(1954) beacaue it was found carcinogenic to humans.

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Not only carcinogenic but the dye also has some other serious harmful effect such as reproductive and developmental toxicity, neurotoxicity, and acute toxicity. It is used in food industries, sweet and confectionary items.

METHODS :-

Preparation of gallic acid calibration curve:

Gallic acid was used as a standard polyphenol to express total polyphenol content in the samples. For this purpose, a gallic acid calibration curve was constructed by taking a range of standard gallic acid concentrations from 0-100µg/ml.

Each of the standard gallic acid solutions were reacted with Folin-ciocalteau reagent sodium carbonate using the above mentioned procedure and absorbance was recorded at 760nm.

Preparation of spices for the extraction:

For the preparation of the extracts, about 100mg from each of the samples was finely crushed and homogenized and dissolved in 1ml of 50% aqueous ethanol(HPLC grade, Merck, Germany).

Then it was taken in graduated tube and centrifuged at 10000g for 5 minutes at room temperature. The supernatant was collected in fresh autoclaved tube and volume was made up to 1 ml with 50% aqueous ethanol.

Preparation of Sodium 10% Carbonate

To prepare 10%(w/v) sodium carbonate solution 1g sodium carbonate(SRL, Mumbai, India)was weighed and dissolved in 6 ml of distilled water.

The solution was shaken vigorously to dissolve the salt completely and volume was finally made up to 10 m.

The extraction of polyphenols

For determination of total polyphenol content, 50µl of plant extract was mixed with Folin-ciocalteau reagent and 750 µl of 10% sodium carbonate solution in a micro centrifuge tube.

All the tubes were wrapped with aluminium foils to avoid exposure to light. The mixture in each tube was shaken well and incubated at room temperature for 30 minutes in dark.

The absorbance was recorded at 760nm in UV-VIS spectrophotometer. Total phenolic content was expressed µg Gallic acid equivalent .

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Chemical test:

Test for rhodamine:

This test was carried out for the whole red chilli and red chilli powder.2 gm of sample was taken in a test tube and to that 5 ml of actone was added. Then the colour change was observed.

Test for rhodamine:

This test was also carried out for the whole red chilli and red chilli powder.½ spoon of the sample was taken in a test tube and to that 3 ml of distilled water was added and then 10 drops of carbon tetrachloride was added. The test tube was shaken vigorously so that the content mixes properly. The red colour disappears. And then dropwise HCL was added.

Test for lead chromate:

This test was carried out for turmeric powder.1 g of sample was taken in a test tube and to that 3 ml of distilled water was added, and then 10 drops of HCL was added.

Test for Sudan III,IV:

This test was carried out for whole red chilli and red chilli powder.1 g of sample was taken in a test tube and to that 2 ml of hexane was added and then it was shaken well. After it was settled down, the upper layer was decanted in another test tube. 2 ml of Aceto nitile reagent was added and then it was shaken well. The colour change was observed0.1 g of sample was taken in a test tube and to that 1 ml of hexane was added and kept to dissolve. After that 5-10 drops of HCL was added to the sample. The colour of the sample was observed.

Test for Metanil yellow:

0.1 g of sample was taken in a test tube and to that 1 ml of hexane was added and kept to dissolve. After that 5-10 drops of HCL was added to the sample. The colour of the sample was observed.

IR spectroscopy

IR spectroscopy consists of the following steps:

About 50mg of KBr was added to 5 mg of the sample and was mixed properly.

After that 7-10 ton(about 8 ton) of pressure is applied manually for a time period of 1 min, then a disc like structure is formed.

And then the absorbance is measured.

Transmittance is changed into the absorbance mode to check the result

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RESULT

$V_1S_1=V_2S_2$
S_1= 1 mg/ml
S_2= (150,300,450,600,750,900)
V_2= 1ml

Table1 : Stock solution preparation of gallic acid:

Sl No.	***V1***	***S1 (mg/ml)***	***V2 (ml)***	***S2 (microgram/ml)***	***Ethanol (ml)***	***Concentration***
1.	0.15	1	1	50	0.85	150
2.	0.30	1	1	100	0.7	300
3.	0.45	1	1	150	0.55	450
4.	0.60	1	1	200	0.4	600
5.	0.75	1	1	250	0.25	750
6.	0.90	1	1	300	0.1	900

Table2 : Optical Density of Gallic Acid :-

Sl No.	***Concentration Of Gallic Acid (microgram/ml)***	***Optical Density(OD)***
1.	150	0.280
2.	300	0.485
3.	450	0.620
4.	600	0.760
5.	750	0.933
6.	900	1.220

Table3 : The absorbance of spices recorded at 760nm:

SPICES	ABSORBANCE

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Whole Black Pepper	0.973
Organic Black Pepper	0.863
Local Black Pepper	0.518
Whole Cinnamon	2.420
Organic Cinnamon	1.998
Local Cinnamon	0.903
Whole Cumin	0.650
Organic Cumin	0.985
Local Cumin	0.828
Whole Turmeric	1.573
Organic Turmeric	0.995
Local Turmeric	0.497
Whole Red Chilli	0.934
Organic Red Chilli	1.085
Local Red Chilli	0.995
Whole Coriander	0.318
Organic Coriander	0.858
Local Coriander	0.620

After plotting the calibration curve, OD vs Concentration , the concentration of root and shoot tissues were found out from the curve by plotting their respective OD.

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Table 5: Spices Vs Concentration

Spices	Concentration
Organic , Local Black pepper	751 , 325
Organic , Local Cinnamon	955, 752
Organic , Local Cumin	820, 655
Organic, Local Turmeric	835, 323
Organic, Local Red chilli	855, 880
Organic, Local Coriander	751, 450

Calculation :-

$$C = C1 * (v/m)$$

C= total phenolic content in microgram/g.

C1= Concentration of gallic acid calculated from the calibration curve or standard curve of Gallic acid in microgram/g.

v = Volume of extract in ml.

m = weight of the spices in gram.

Total phenolic content of

Organic Black pepper is 0.03004 microgram/g. **Organic Cinnamon** is 0.0382 microgram/g. ; **Organic Cumin** is 0.0328 microgram/g. ; **Organic Turmeric** is 0.0374 microgram/g ; **Organic Red chilli** is 0.0342 microgram/g. ; **Organic Coriander** is 0.03004 microgram/g. ; Local Black pepper is0.0130 microgram/g. ; **Local Cinnamon** is 0.03008 microgram/g. ; **Local Cumin** is 0.0262 microgram/g.**Local Turmeric** is 0.01292 microgram/g. ; **Local Red chilli** is 0.0352 microgram/g. ; **Local Coriander** is 0.0180 microgram/g

Table**4: CHEMICAL TEST :-**

Spice	Adulterant	Rapid test	Observation	Interference
Whole red chilli & red chilli powder	Rhodamine	2 gm of sample was mixed with 5 ml of acetone		Immediate appearance of red colour in red chilli powder indicates the presence of dye

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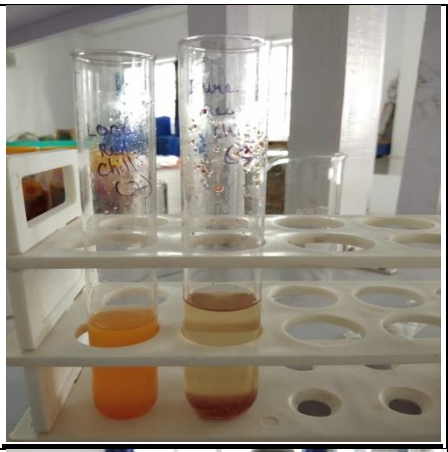
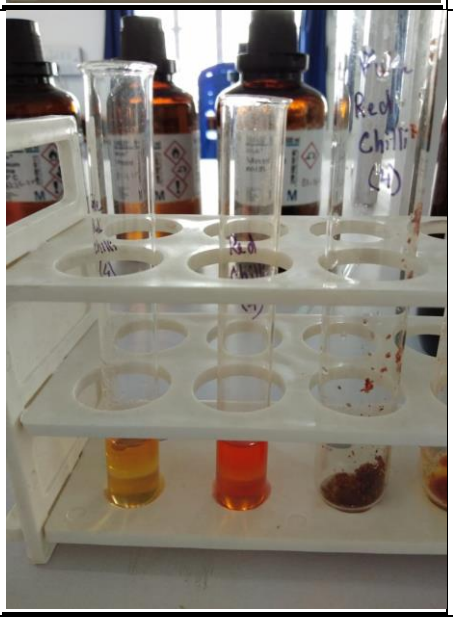
Whole red chilli & chilli powder	Rhodamine	½ spoon of sample was mixed with 3 ml of distilled water. Test tube was shaken vigorously		The reddish appears after addition of HCL, indicates the presence of dye
Whole red chilli & red chilli powder	Sudan III,IV	1 gm of sample +2ml of hexane. Upper layer was decanted and 2 ml of acetonitrile reagent was added and shaken		Appperance of red color after addition of acetonitile reagent indicates the presence of dye

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Turmeric powder	Lead chromate	1g of sample+ 3 ml of distiiled water and 10 drops of HCL		The absence of pink color indicates the the absence of lead chromate in the sample.

Conclusion

From the experimental work, it is seen that red chilli contains the highest amount of antioxidant. Cinnamon and red chilli also contains huge amount of antioxidants. It is better to use the whole form of spice or the organic ones as they are less likely to contain unpermitted dyes which are not only harmful but also carcinogenic. Spices should ben important part of our life and to be consumed not only for their taste but also for their enormous health benefits.

From the absorbance it can be said that the organic form of the spice contains more antioxidant than the other forms and so is the purity. From the chemical test it is confirmed that the local powder form of the spice contain dyes like rhodamine and sudan III,IV, where as the local turmeric powder is free from lead chromate, which is dangerous in nature, though there is a possibility of the presence of other dyes in minor amount.

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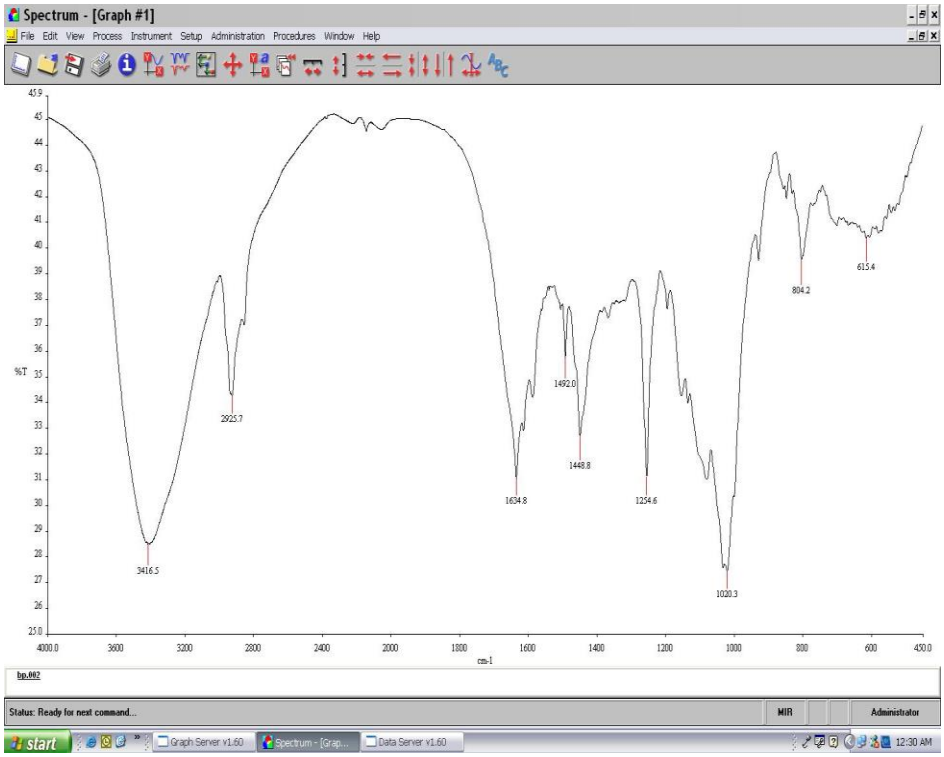
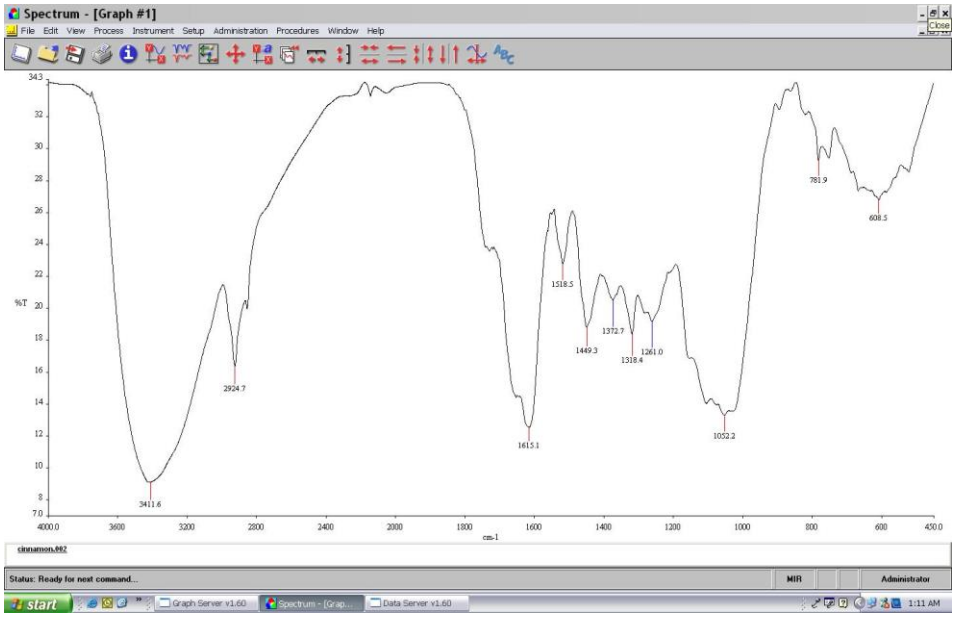
5. Infrared Spectrometry observation :-

5.1 IR Graph of Black pepper :-



5.2 IR Graph of Organic Cinnamon :-



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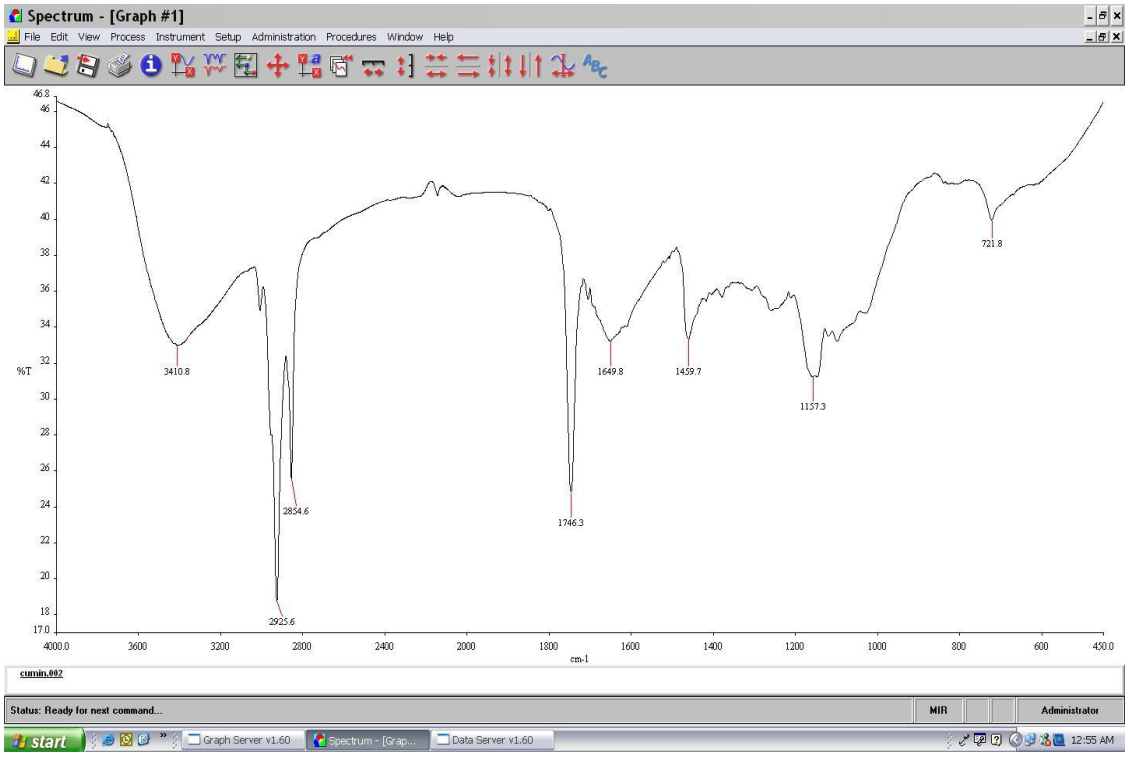
5.3 IR Graph of Organic Cumin :-



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***ISSN*2687-7939 (Online)**