

**QUANTITATIVE AND QUALITATIVE ANALYSIS OF PHYTOCHEMICAL AND
ANTIOXIDANT PROPERTIES OF CINNAMOMUM VERUM (DALCHINI)**

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ABSTRACT

Bark of cinnamon is powdered and solvent is extracted by using hydroalcohol. It is rich in antioxidant and phytochemical constituent which are useful in pharmaceutical industry for drug formulation as well as cosmetic industry. TLC is done to identify the number of compounds present in cinnamon and Doboy's method is used to determine the total carbohydrate estimation and Laury method is used for protein estimation. DPPH is used to predict the free radical-scavenging property of cinnamon. Based on the results, the ethanol extracts of Cinnamon barks have potential value as an antioxidant substitute and this study also provide a better technique to extract the natural antioxidant substances from *C.verum*.

KEYWORDS: Thin layer chromatography, DPPH, antioxidant, phytochemicals.**INTRODUCTION**

India is rich in various species which we use in our day to day life like cinnamon, black pepper, turmeric, bay leaves etc. All species makes our food tasty and play an important role in our body by strengthening our immunity. They also contain antioxidant, antimicrobial, antifungal and anti inflammatory properties which are responsible for a healthy body functioning and also increase metabolic activity. Cinnamon is one them which acts as both spices and medicine. *Cinnamomum verum* belongs to family Lauraceae. In India cinnamon is mainly cultivated in Western Ghats of Kerala and Lower Nilgiris of Tamil Nadu. According to Sanskrit it is famous with the name "Dalchini".^[1]

Today scientists found their interest in natural and organic products to make different kinds of medicine. When they study about phytochemical properties of cinnamon (*Cinnamomum verum*) they predict that it has various pharmacological properties for example- they control diabetes, they have antioxidant property which helps to prevent cancer, they also prevent urinary tract infections (UTI) due to their antimicrobial activity.^[2] Cinnamon is also a best remedy for respiratory, digestive and gynaecological ailments according to native ayurveda. The powder of cinnamon bark is in high demand in cosmetic industries also. It also helps to make our heart healthy by reducing the bad cholesterol and stabilizing the good cholesterol thereby prevents heart attacks.^[3] Different types of facial creams, face packs contain cinnamon which help in breakouts and also controls acne and pimples which are caused due to some

bacteria and fungus. If it is applied on hair it also promotes hair growth and prevent fungal infections such as dandruff. The main objective of this study is to predict the phytochemical properties quantitatively and qualitatively and antioxidant properties of *Cinnamomum verum*.

2 MATERIALS AND METHODS**2.1 Collection of Sample**

Barks of *Cinnamomum verum* were collected from local areas of Bhopal, Madhya Pradesh between May to June 2019 and crushed into fine powder.

2.2 Preparation of Plant Extract with Distilled Water and Ethanol

50gm of cinnamon bark powder is weighed and added 100ml of ethanol and 100ml of distilled water (i.e. hydroalcohol is used for extraction) stirred it constantly for every 30 minutes and the prepared solution is kept at room temperature for 24 hrs to 1 week.

3. THIN LAYER CHROMATOGRAPHY

TLC can be used to help determine the number of components in a mixture, the identity of compounds, and the purity of a compound. Normal phase silica gel pre-coated TLC plates were used.

Methanol and water (3:7) used as a solvent system. 1 drop solution of cinnamon extract was placed about 1.5cm on silica plates using micropipette. The plates were placed vertically into the solvent system. When the solvent system moved 80% from the spotting line, the

plates were moved from the developing chamber and dried. When the plates become dried completely it was observed under the UV transilluminator to see the bands.

4. ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS

4.1 Alkaloids: Wagner's Reagent

1 ml of cinnamon extract is taken in a test tube and 2% H_2SO_4 is added and warm for 2 minutes in water bath. After that add few drops of Wagner's reagent.

Reddish brown precipitate indicates the presence of alkaloids.

PREPARATION OF WAGNER'S REAGENT: 2.5gm of iodine is dissolved in 12.5gm of potassium iodide. 250 ml of water is added to produce the solution.

4.2 FLAVANOIDS

1ml of extract was taken; 2 ml of ethyl acetate is added and warm in water bath for 3 minutes.

1ml of 1% ammonia solution is added and shakes well.

The layer allowed to separate yellow colour which is observed in ammonia layer indicates presence of flavanoids.

CONFIRMATORY TEST

1ml of extract is taken and 1ml of 1% ammonia chloride is added and shakes well. Observation of light yellow colour gives positive result for flavanoids.

4.3 TERPENOIDS

1ml of extract is taken in test tube, 2ml of chloroform and 1ml of concentrated H_2SO_4 is mixed to the extract.

Reddish brown colour of interphase indicates the presence of flavanoids.

4.5 PHENOL

1ml of extract is taken in a test tube 1ml of lead acetate is added. Fumy white colour gives positive result for phenol.

Confirmatory Test

1ml of extract is taken, 1ml ethanol and 1ml 5% perichloride is added to it.

Greenish black blue colour confirms the presence of phenol.

4.6 Steroid

1ml of extract is taken, 2ml acetic acid and 2ml of H_2SO_4 is mixed to it. Colour change from violet to blue green indicates the presence of steroid.

4.7 Saponins

1ml of extract is taken in a test tube and 4ml distilled water is added, shake vigorously stable form is observed which gives the positive result for saponins.

4.8 Carbohydrate

1ml of extract is taken few drops of Molisch reagent and 1ml of concentrated H_2SO_4 is added. Formation of brown ring at the interphase indicates presence of carbohydrates.

5. QUANTITATIVE ESTIMATION

5.1 Phenol

0.5ml of extract is taken and 1.5ml distilled water is added, 1ml of folin's reagent (1:9) is added to it.

Allow to stand for 5minutes.

After 5 minutes, add 2ml 7.5% sodium carbonate. Put the mixture for incubation for 90 minutes at dark. After 90 minutes take O.D on colorimeter with blue filter.

5.2 Flavanoid

0.5ml of extract is taken and 1.5ml of distilled water and 0.5ml of sodium nitrite is added. Allowed to stand for 6 minutes then 0.5ml 10% aluminium trichloride is added.

Incubate for 6 minutes.

2ml 4% sodium hydroxide is added later and incubates for 10 minutes again.

Take O.D at 510 nm.

5.3 Alkaloids

1ml of extract is taken and 0.5ml of methanol is added and 1ml 2N HCL is added. Neutralise the solution with 0.1N NaOH. 2ml of BCG and 5ml phosphate buffer was added. The mixture was shaken properly by adding 4ml chloroform. The absorbance is taken at 470nm.

5.4 Carbohydrate Estimation: (Doboy's Method)

The dilution of glucose is made from 100 to 500 dilutions and used as standard.

0.5ml extract was taken and the volume was made the volume up to 2ml in distilled water.

2ml concentrated H_2SO_4 and 1ml of 5% phenol solution was added in each test tube. Distilled water, phenol and H_2SO_4 was taken in one test tube and used as a blank.

Incubate for 20 minutes at 30 °C. Take O.D at 490nm.

5.5 Protein Estimation: (Laury Method)

REAGENT A- 2% sodium carbonate was mixed in 50ml 0.1N sodium hydroxide.

REAGENT B- 0.5% $CuSO_4$ was mixed in 10ml 1% sodium tartarate.

REAGENT C- Mix 1ml of reagent B in 50ml of reagent A.

REAGENT D- Folin's reagent was mixed with distilled water in 1:1 ratio.

The dilution of egg albumin was prepared from 200 μ L to 1000 μ L and make up the volume up to 1ml with distilled water.

5ml of Reagent C was added and incubates for 10minutes. 0.5ml of Reagent D was added and incubates for 30 minutes.

After 30 minutes O.D was taken at 660nm.

6. ANTIOXIDANT PROPERTIES

6.1 Nitric oxide free radical scavenging activity

REAGENTS

- 5mM sodium nitroprusside
- Phosphate buffer 7.4 pH
- Griess reagent (1% sulphanilamide, 0.1% naphthylethylenediamine dihydrochloride, 2% orthophosphoric acid).

The dilution of plant extract was made up to 100 μ L -500 μ L. The dilution of L-ascorbic acid was prepared up to 100 μ L -500 μ L and used as standard. 1ml of sodium nitroprusside was added in each test tube. Incubate at room temperature for 150minutes. 1ml of griess reagent is added in each test tube and O.D was taken at 550nm. Distilled water, sodium nitroprusside and griess reagent was taken in one test tube and used as blank. The percentage scavenging of nitric oxide of plant extract and standard solution of potassium nitrite is calculated using the following formula:

$$\text{NO Scavenged (\%)} = (\text{Ac}-\text{Ae})/\text{Ac} \times 100$$

Where Ac = Absorbance of control reaction and Ae = Absorbance in presence of sample.

6.2 Ferric reducing anti-oxidant power assay

The dilution of plant extract was made up to 100 μ L -500 μ L.

The dilution of L-ascorbic acid was prepared up to 100 μ L -500 μ L and used as standard.

2.5ml of 20mM phosphate buffer and 2.5ml 1% potassium ferricyanide was added. Incubate the mixture at 50°C for 30 minutes. After 30minutes 2.5ml 10% TCA and 0.5ml 0.1% ferric chloride was added. Incubate for 10 minutes at room temperature and O.D was taken at 700nm.

6.3 DPPH Assay: DPPH. (2, 2-diphenyl-1-picrylhydrazyl)

0.04mM DPPH was prepared in ethanol. 100 μ L extract dissolved in 900 μ L methanol. 3 test tubes were taken and 1ml methanol and 1ml DPPH, 1 ml ascorbic acid and 1ml DPPH, 1ml plant extract and 1ml DPPH was added

in each test tube respectively. Incubate for 30 minutes at room temperature. O.D was taken at 550nm. Ethanol was taken as zero.^[4]

7. RESULT AND DISCUSSION

With the help of phytochemical analysis we are able to know that different phytoconstituents like alkaloids, flavanoids, terpenoids, phenol, carbohydrate, steroid, saponins, and carbohydrates are present in *Cinnamomum verum*. Phytochemical constituents contain pharmacological and toxic activities in plant, which are sometimes only useful to plant itself and toxic to animals including humans.

7.1 Phytochemical Analysis

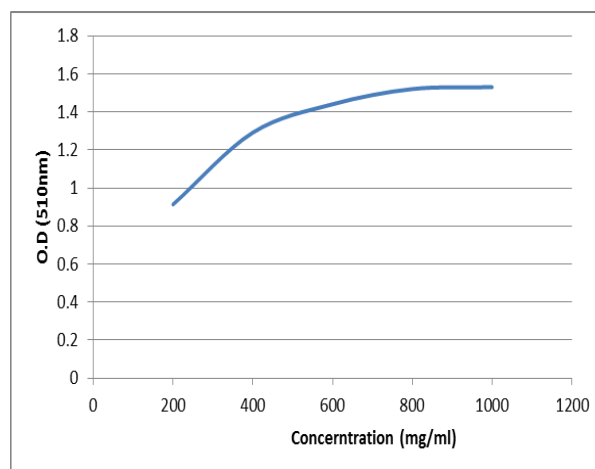
Table 1.1: From the above table (1.1) we found that in *cinnamomum verum* alkaloids, flavanoids, terpenoids, phenol saponins and carbohydrate are present and steroid is absent.

	<i>Cinnamomum verum</i>
Alkaloids	+ve
Flavanoids	-ve
Terpenoids	+ve
Phenol	+ve
Steroid	-ve
Saponins	+ve
Carbohydrate	+ve

7.2 QUANTITATIVE ANALYSIS

7.2.1 Total Phenol Estimation

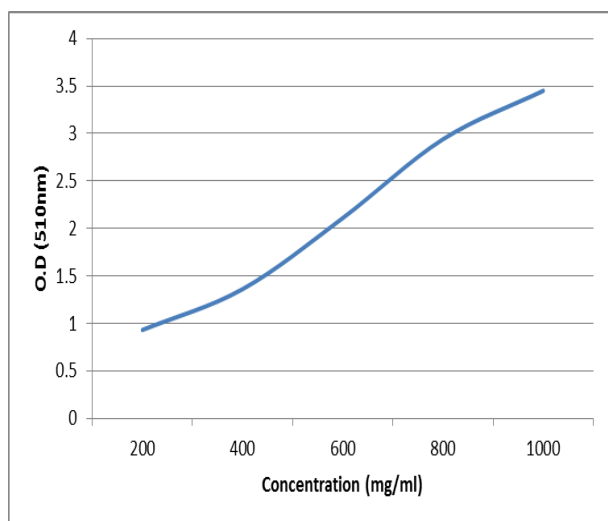
Plants contain different types of secondary metabolites phenol is one of them. Phenolic compounds are water soluble in nature. Due to presence of phenolic compounds species are able to produce aroma, test, colour, flavour and pungency. The total phenol content found to be 776.32mg/ml of tannic acid equivalent in hydro alcoholic extract of *C.verum*. Similar work was done by Vincenta Khristi et.al in 2014 by Folin Ciocalteu method and they found total phenolic content in cinnamon aqueous extract was found to be 481 mg GAE/100g.^[5]



Graph 1.1 Standard Curve of Tannic Acid.

7.2.2 Total Flavanoid Content

The total flavonoids, such as flavanols, flavonols, isoflavones, and anthocyanidins, have been reported to have multiple biological effects, including antioxidant activity. The capacity of flavonoids to act as antioxidants depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. The total flavanoid content found to be 388.76mg/ml of tannic acid equivalent in hydro alcoholic extract of *C.verum*. The same experiment was done by the Cheng-Hong Yang et.al in 2012 the total flavanoid content of the ethanol extracts barks found to be 2.030 g/100 g DW.^[6]

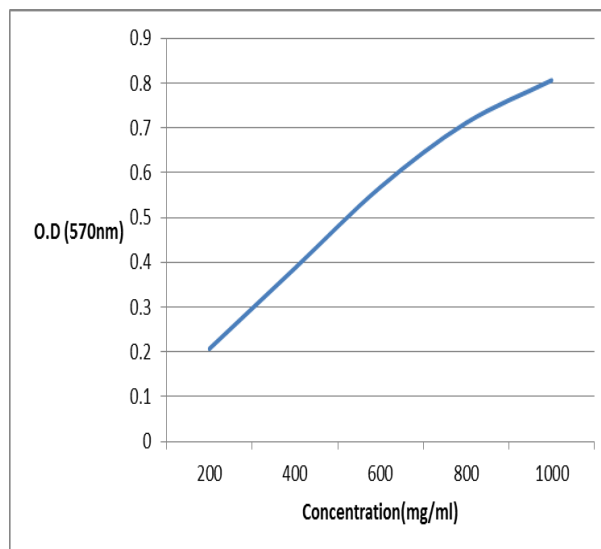


Graph 1.2: Standard curve of tannic acid.

7.2.3 Total Alkaloid Content

The proposed roles of alkaloids in plant metabolism, plant catabolism, or plant physiology are (1) end products of metabolism or waste products, (2) storage reservoirs of nitrogen, (3) protective agents for the plant against attack by predators, (4) growth regulators (since structures of some of them resemble structures of known growth regulators), or (5) substitutes for minerals in plants, such as potassium and calcium.

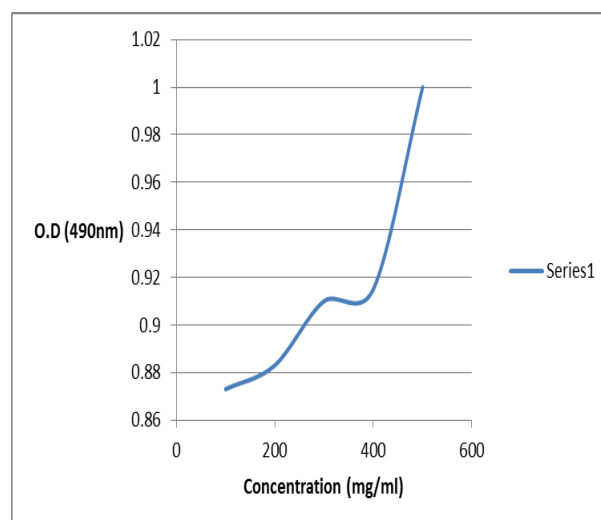
The total alkanoid content found to be 755.93mg/ml of tannic acid equivalent in hydro alcoholic extract of *C.verum*. Similar work was done by Siddhartha Eadlapalli et.al in 2016 by same method but he took methanolic extracts instead of methanolic and he found 0.70mg AE/g.^[7]



Graph 1.3: Standard curve of tannic acid.

7.2.4 Total Carbohydrate Estimation

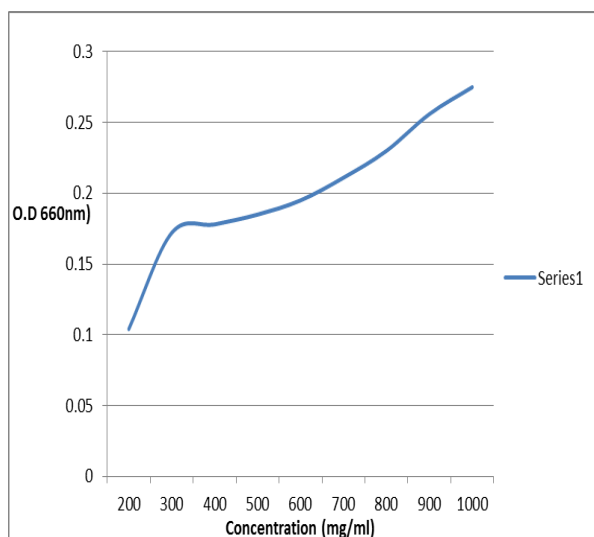
The total carbohydrate content found to be 310.8346mg/ml of glucose equivalent in hydro alcoholic extract of *C.verum*.



Graph 1.4: Standard curve of glucose.

7.2.5 TOTAL PROTEIN ESTIMATION

The total protein content found to be 1776.245mg/ml of egg albumin equivalent in hydro alcoholic extract of *C.verum*.

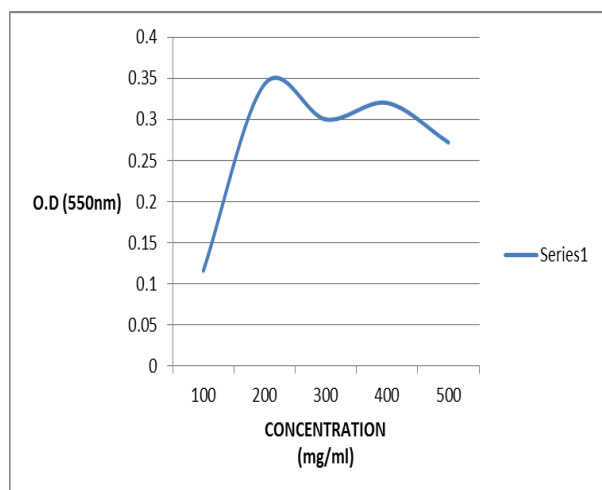


Graph 1.5: Standard curve of egg albumin.

7.3 ANTIOXIDANT

7.3.1 Nitric oxide free radical scavenging activity^[8]

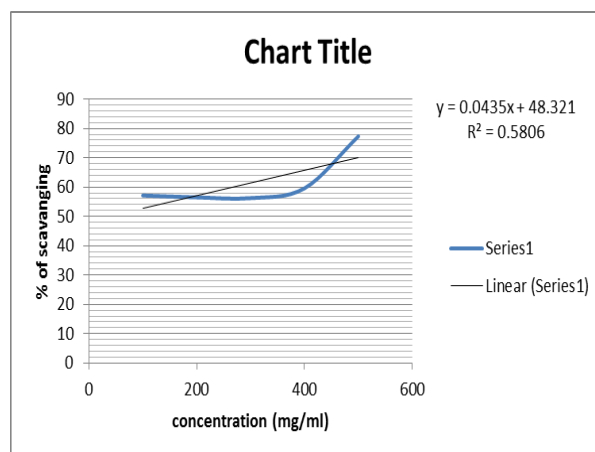
Nitric oxide (NO) is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, and inhibition of platelet aggregation and regulation of cell mediated toxicity. In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions. NO is known to be a ubiquitous free-radical moiety, which is distributed in tissues or organ systems and is supposed to have a vital role in neuromodulation or as a neurotransmitter in the CNS.



Graph 1.6.

7.3.2 Ferric reducing anti-oxidant power assay

In this study, the reducing capacity of the extracts and fractions were performed using Fe³⁺ to Fe²⁺ reduction assay. In this experiment, the yellow color changes to pale green and blue color depending on the concentration of antioxidants in the samples. The antioxidants such as phenolic acid and flavonoids were present in considerable amount in the extract of *c.verum*. This method is known as FRAP.^[9]



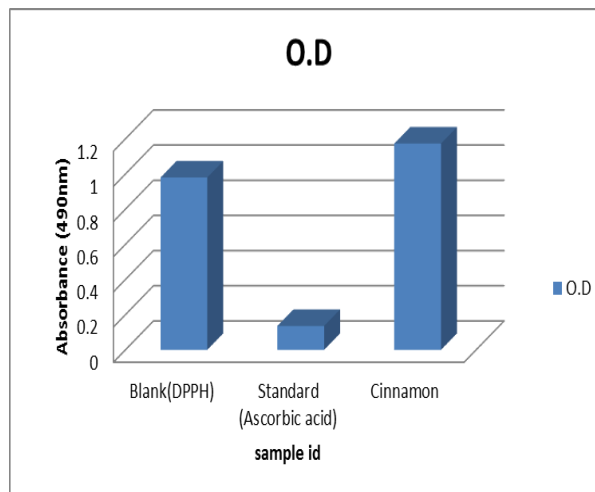
Graph 1.7.

IC50 VALUE = 39.069

The IC₅₀ value defined as the concentration of the sample required for 50% scavenging of ferric reducing power was calculated. It is inversely proportional to greater potency and from our result it is clear that the bark extracts have greater potency of ferric reducing.^[10]

7.3.3 DPPH Assay: DPPH. (2, 2-diphenyl-1-picrylhydrazyl)

Inhibition of DPPH radical was measured as the decrease in absorbance of the samples versus DPPH standard solution. Lower absorbance of the reaction mixture indicated higher free radical-scavenging.



Graph 1.8: From the graph 1.8 it is clear the ascorbic acid show less absorbance than cinnamon extract so it has the capacity of higher free radical- scavenging.

CONCLUSION

Now a day different types of disease are increasing at high rate which are very harmful to the humans. From the study it is identified that Cinnamon is rich in antioxidant properties which helps in preventing cancer. Due to its antiseptic properties it is used in cosmetic industries. It is also demanded in pharmaceutical industries for the preparation of anti-diabetic, anti-inflammatory, antimicrobial drugs. Therefore, Cinnamon

plays an important role in modern system of medicine as a multipurpose medicinal spice.

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