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PHYTOCHEMICAL SCREENING OF MARKET SAMPLES OF TVAK (CINNAMOMUM ZEYLANICUM BREYN.)

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ABSTRACT

Phytochemicals are the natural bioactive products such as Tanins, Alkaloids, Carbohydrates, Terpenoids, Steroids, Flavonoids, Sapponins etc. These bioactive compounds founds in different parts of plants such as leaves, root, bark, fruits, flower, etc. Medicinal plants are the source of naturally active compounds. Cinnamon is very well known herb, which is used to treat many aliments from ancient era. Cinnamon is pretty famous in pharmaceutical industry, food industry. Phytochemical are widely used in human therapy, veterinary, agriculture, scientific research and countless fields. Extraction of bioactive plant constituents from whole plants or part of plant has always been challenging task. As bark of Cinnamon tree is widely used in the treatment of many diseases. Current study aimed at quantitative and qualitative analysis of phytochemicals from different extracts of Cinnamon bark powder.

Keywords: Phytochemicals, bioactive compounds, Cinnamon

INTRODUCTION

Since very ancient period interest has been revived in study and use of traditional medicine in different parts of world. On regarding this different countries are supporting and promoting the use of traditional medicine in their national health system.

From vedic period people have been exploring the various herbal drugs their uses formulations. In India almost 70% of prescription consist of herbal medicine.[1] In traditional systems of medicine such as Ayurveda , Homeopathy, Unani, Siddha research is on its progressive track. Plants produce primary and secondary metabolites with divergent function. The primary, metabolites are amino acids, proteins, lipids. Secondary metabolites are bioactive products such as alkaloids, glycosides, flavonoids etc. These secondary metabolites brings out their effects on other organisms.[2]

Tvak i.e. Cinnamomum zeylanicum is commonly known as Cinnamon. It is an evergreen tree having pale around 16cm thick bark. Inner portion of it is red colored. Leaves are like leather and hairy.[3] They have shiny dorsal aspect with 3 to 5 veins on them. Flower

are dusky colored, fruits are violet in color 12 cm long. Tree bears flowers and fruits in spring. Cinnamon is a member of Lauraceae family. Main source of cinnamon is Shrilanka, Burma, China. In India: Southern India and Himalaya[4]. Mainly the tree bark and leaves are used in formulations such as *Sitopaldi churna, Talisadi churna, Lavanbhaskar churna, Chandraprabha vati* etc.[5]

Cinnamon comprises of Dipana and Pachana karma. It is used in treatment of various diseases such as *Aruchi, Agnimandya, Kasa, Pinas, Kanthroga, Mukharoga, Hridriga, Krimi* etc.[6] it also shows antiinflamtory, antimicrobial, antibacterial, antifungal, type-2 DM, activity.[7,8,9,10]

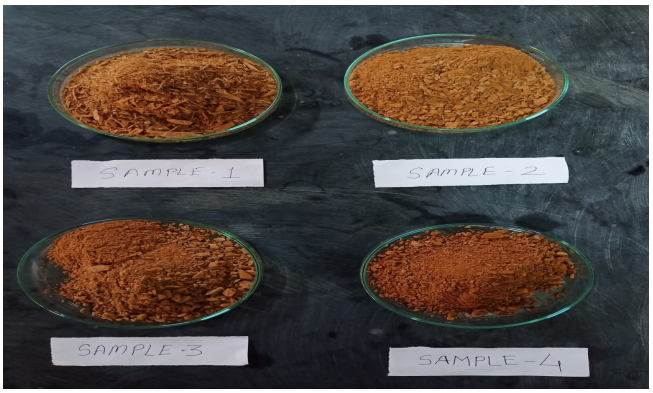
In order to promote the use of herbal medicines it is very needful to perform screening of plants for secondary metabolites.

Many other studies includes pharmacological evaluation of cinnamon bark, here present srtudy deals with the four different market samples of cinnamon collected from different areas. In market there are more than 10 to 20 varieties of cinnamon are available. So it is necessary to perform phytochemical analysis in order to its authentication and standardization, because efficacy of any pharmaceutical formulations is depends on its purity.

MATERIALS AND METHODS:

Market samples of cinnamon were collected from reliable market source. And it authenticated by a botanist. Following are 4 market samples;

1) Cinnamomum zeylanicum
2) Cinnamomum burmani
3) Cinnamomum laureirai
4) Cinnamomum laireirii



Methods of Alkaloid and Tannins Estimation[11]

Table 1: Sample preparation

SAMPLE NO.	Quantity of sample powder taken	Methanol Taken	Methanolic extract (in ml)
1	5gm	25 ml	4ml
2	5gm	25 ml	5ml
3	5gm	25 ml	11ml
4	5gm	25 ml	11ml

ALKALOID ESTIMATION

To Estimate Total Alkaloids in Plant Extracts

Principle: Bromocresol green (BCG) reacts with alkaloids having nitrogen atom within ring and forms yellow colored complex which can be easily measured by using colorimeter. BCG doesn't reacts with alkaloids having nitrogen inside chain, and thus, this method is not useful to determine amine or amide alkaloids.

Reagents

- **BCG Solution** (Dissolve 69. 8 mg BCG in 3 ml 2N NaOH and 5 ml distilled water. Make up the volume up to 100 ml),
- Phosphate Buffer Solution (pH 4.7), and
- **Standard Atropine Solution** (Dissolve 1 mg atropine in 10 ml distilled water).

Procedure

1. Take 0.4. 0.6, 0.8, 1.0, and 1.2 ml atropine solution in a separate test tube.
2. Add 5 ml of Phosphate Buffer Solution (pH 4.7) and 5 ml of BCG solution.

3. Shake well and extract the yellow colored complex with chloroform.
4. Separate chloroform and make up the volume to 10 ml.
5. Measure the absorbance at 470 nm against blank.
6. Now prepare the methanolic extract of plant material. Dry and dissolve in 2N HCl. Filter and wash with chloroform. Adjust the pH neutral with 0.1 N NaOH.
7. Now add 5ml of Phosphate Buffer Solution (pH 4.7) and 5 ml of BCG solution.
8. Shake well and extract the yellow colored complex with chloroform.
9. Separate chloroform and make up the volume to 10 ml and measure the absorbance at 470 nm.
10. Calculate concentration of total alkaloids from calibration curve of atropine standard.

Table 2: Standard Atropine

Sr. No.	Standard Atropine	Absorbance
1.	0.4 ml	0.044
2.	0.6ml	0.047
3.	0.8ml	0.050
4.	1.0ml	0.054
5.	1.2ml	0.058

Table 3: Sample Readings:

SAMPLE NO.	Absorbance
1	0.089
2	0.050
3	0.048
4	0.054

Calculations:

Y=0.003x+0.040

1. for sample 1

Y=0.089

0.089 = 0.003x + 0.040

x=0.089-0.040/0.003

x=16.33 mg/ml

=65.32 mg/4ml

5 gm sample contains 65.32mg of alkaloid

100 gm will contain - 100 x 65.32/5

=1306.4mg/g

=1.306 %

2. for sample 2

Y=0.050

0.050 = 0.003x + 0.040

x=0.050-0.040/0.003

x=3.33mg/ml

x=16.65mg/5ml

5 gm sample contains 16.65mg of alkaloid

100 gm will contain –100 x 16.65/5

=333mg/g

=0.33%

3. for sample 3

Y=0.048

0.048 = 0.003x + 0.040

x =0.048-0.040/0.003

x=2.66mg/ml

x=29.33mg/11ml

5gm sample contains 29.33mg of alkaloid

100 gm will contain – 100 x 29.33/5

=586.66 mg/g

=0.586 %

4. for sample 4

Y=0.054

0.054 = 0.003x + 0.040

X = 0.054-0.040/0.003

X=4.66mg/ml

X=51.26mg/ml

5gm sample contains 51.26mg of alkaloid

100 gm will contain – 100 x 51.26/5

=1025.2mg/g
=1.025 %

Tannins Estimation By Folin Denis Method:
To Estimate Total Tannins by Folin-Denis Reagent
Principle: Tannins are the polyphenolic compounds which can be classified as hydrolysable and non-hydrolysable (condensed tannins). Citrus fruits, red wine, and tea leaves are the important source of natural tannins. They have the property to bind and precipitate proteins. Tannins reduce phosphotungstomolybdic acid in alkaline solution to produce a highly colored blue solution, the intensity of which is proportional to amount of tannins. The intensity is measured in a spectrophotometer at 700nm.

Reagents

- **Folin-Denis Reagent** (Dissolve sodium tungstate (10g) and phosphor molybdic acid (2g)in distilled water (75ml)along with phosphoric acid(5ml).Reflux the mixture for 2 hours and make up the volume with water up to 100ml),
- **Sodium Carbonate Solution** (Dissolve sodium carbonate (35g) in distilled water (up to 100ml). Allow to stand overnight and filter through glass-wool), and
- **Working Standard Solution of Tannic Acid** (Dissolve accurately weighed tannic acid (100 mg) in distilled water and make up the volume to 100ml in volumetric flask. Dilute 5ml of this solution with water to 100ml in another volumetric flask to give 50g/ml tannic acid solution).

Procedure

1. Take 1, 2, 3, 4 and 5 ml working standard solution of tannic acid in a separate test tube.
2. Add 0.5 ml of Folin-Denis reagent and 1ml sodium carbonate to each test tube.
3. Make volume of each test tube up to 10 ml. Blue color solution is formed. Measure the absorbance at 700 nm within 30 min against their agent blank prepared in a similar manner without the tannic acid.
4. Calculate the percentage of total tannin from calibration curve of tannic acid.

Table 4: Standard Tannic Acid

Sr. No.	Tannic acid Std.	Absorbance
1.	1 ml	0.108
2.	2ml	0.229
3.	3ml	0.313
4.	4ml	0.365
5.	5ml	0.493

Table 5: Sample Reading:

Sample No.	Absorbance
1	0.656
2	0.494
3	0.376
4	0.517

CALCULATIONS
Y=0.090x+0.029
1. for sample 1
Y=0.656
0.656=0.090x+0.029
x=0.656-0.029/0.090
x=6.97 mg/ml
=27.86 mg/4ml
5 gm sample contains 27.86 mg of alkaloid
100 gm will contain- 100 x 27.86/5
=557.33 mg/g
=0.557 %
2. for sample 2
Y=0.494

0.494=0.090x+0.029
x=0.494-0.029/0.090
x=5.17mg/ml
=25.83 mg/5ml
5 gm sample contains 25.83mg of alkaloid
100 gm will contain- 100 x 25.83/5
=516.6 mg/g
=0.516%

3. for sample 3

Y=0.376
0.376=0.090x+0.029
x=0.376-0.029/0.090
x=3.86 mg/ml
=42.46 mg/11ml
5 gm sample contains 42.46 mg of alkaloid
100 gm will contain- 100 x 42.46/5
=849.2 mg/g
=0.849 %

4. for sample 4

Y=0.517
0.517 = 0.090x+0.029
x=0.517-0.029/0.090
x=5.43 mg/ml
=59.73 mg/11ml
5 gm sample contains 59.73 mg of alkaloid
100 gm will contain- 100 x 59.73/5
=1194.6 mg/g
=1.194 %

OBSERVATION:

Table No. 6

TESTS	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4
1.TEST FOR CARBOHYDRATES				
Molisch test	+	+	+	+
Test for reducing sugars				
Fehlings test	+	+	-	+
Benedicts test	+	+	-	+
Test for monosaccharides				
Barfoeds test	+	-	-	+
2.TEST FOR PROTEINS				
Biuret test	-	-	-	-
Millon's test	-	-	-	-
3. TEST FOR AMINO ACIDS				
Ninhydrin test	-	-	-	-
4.TEST FOR ALKALOIDS				
Dragendroff's test	+	+	+	+
Mayer's test	+	+	+	+
Wager's test	+	+	+	+
5.TEST FOR CARDIAC GLYCOSIDES				
Legal's test	-	-	-	-
Keller-Killani test	-	-	-	-
6.TEST FOR ANTHRAQUINONE GLYCOSIDE				
Brontrager's test	-	-	-	-
7.TEST FOR SAPONINS				
Foam test	-	+	+	+
8.TEST FOR STEROIDS				
Salkowaski test	-	-	-	-
9.TEST FOR FLAVONOIDS				
Shinoda test	-	-	-	-
Sulphuric acid test	-	-	-	-
10.TEST FOR TANNINS				

5%$FeCl_3$ solution	+	+	+	+
Lead Acetate	+	+	+	+
Potassium dichromate	+	+	+	+

'+'indicates positive test results '-'indicates negative test result

Table 7: ALKALOID CONCENTRATION:-

SAMPLE NO.	Alkaloid concentration
1	1.306%
2	0.33%
3	0.586%
4	1.025%

Table 8: Tannins Concentration:-

Sample No.	Tannins concentration
1	0.557%
2	0.516%
3	0.849%
4	1.194%

RESULT & DISCUSSION

As cinnamon is very well known between common people, food and drug industry it is necessary to standardize it, due its increasing demand chances of adulteration is also increasing. All the four samples were collected from different parts of India, having different morphological characters. Phytochemicals are secondary metabolites derived from plants. All the four samples went though both qualitative and quantitative analysis.

Qualitative tests were done for carbohydrates, reducing sugars, monosaccharides, proteins, amino acids, alkaloids, cardiac glycosides, antraquinone glycosides, saponins, steroids, flavonoids, tannins. Carbohydrates are present in all the four samples.

Reducing sugar is found in 1st, 2nd and 4th sample. It is any sugar that is capable of acting as reducing agent because it has free aldehyde group or free ketone group.[12] All **monosaccharides** are reducing sugars, along with some disaccharides, oligosaccharides and polysaccharides[13]. It is carbohydrates that are oxidize by weak oxidizing agent. Proteins, Amino acids, Cardiac glycocides, Anthraquinone glycocides, steroids, flavonoids are absent. Alkaloids and Tannins are present in all four samples.

Alkaloids are metabolic product in plants. They play very important role in organism metabolism and functional activity. They play important role in immune system of animals and plants. Alkaloids have diverse and important physiological effects on human and animals.[14]

Tannins generally possess an astringent flavor and activity, which relates to their ability to indiscriminately bind proteins. Tannins draw tissues together as proteins congeal, causing peculiar puckering sensations in mouth. The tannins compounds are widely distributed in many species of plants, where they play a role in protection from predation and might help in regulating plant growth.[15]

CONCLUSION

The preliminary phytochemical analysis of methanolic extracts of all the four samples shows the presence of Carbohydrayes, alkaloids, saponins, tannins. So the quantitative analysis shows that more alkaloid presence in sample 1 (1.306%) and less in sample 2 (0.33%). And more amount of tannin is found in sample 4 (1.194 %) and less in sample 2 (0.516%).

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