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Evaluation of antimicrobial and antioxidant property of Kalmi: Dalchini

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Abstract
Purpose: Evaluation of antimicrobial and antioxidant property of kalmi dalchini precisely called as cinnamon zeylanicum. The cinnamon extract be collected and phyto cemical tests are conducted. Evaluation of antimicrobial and antioxidant property done by *in vitro* process.
Methods: The essential oil be carried out by using the soxhlet extraction. Its properties are identified by the various methods such as agar streak dilution method, serial dilution method, agar diffusion and by turbidimetric methods. Antioxidant studies as analysed by hydrogen peroxide scavenging test.
Results: Generally ciprofloxacin effects on gram positive bacteria. The extract sample compared by standard ciprofloxacin, as the concentration of ciprofloxacin increases zone of inhibition increases.
Conclusion: Cinnamon zeylanicum is a traditional medicine. The most important constituent cinnamic acid and cinnamaldeydes present in the bark are responsible for its antimicrobial activity, and antioxidant activity is due to the presence of ascorbic acid.

Keywords: Antimicrobial and antioxidant, Kalmi

Introduction
Cinnamon (*Cinnamon zeylanicum*) also called as dalchini is the eternal tree of tropical medicine which belong to class lauaracea, is native to srilanka and malabar coast, The botanical name cinnamomum is derived from hebraic and arabic term amomon, meaning fragrant spice plant [1]. The bark of dalchini is one of the most popular spices used worldwide not only for cooking but also in traditional and modern medicine [2]. Cinnamon is mainly used in the aroma and essence due to its fragrance. The most important constituent of cinnamon are cinnamaldehyde and Trans cinnamaldehyde which present in essential oil [3].
Dalchini used as a coagulant and prevent bleeding [4], It plays vital role as anti- oxidant, Antifungal [7] and as Antimicrobial agent, It has been used as Anti-inflammatory and also as Anti carcinogenicagent [8]. Cinnamon consist of variety of resinous compounds including cinnamaldehyde, cinnamate, cinnamic acid and essential oils, Its fragrance is due to presence of cinnamaldehyde [9, 10] Dalchini had a wide range of antimicrobial activity, The antimicrobial activity is due to cinnamaldehyde. This cinnamic acid inhibit the growth of both gram positive and gram negative food borne pathogens, Cinnamon bark is rich in cinnamaldehyde it contains nearly 50.5% of cinnamaldehyde which is highly electronegative and interferes in biological process involving electron transfer and react with nitrogen containing compounds, Flavonols present in the bark also plays vital role in antifungal activity [11]. The Antioxidant activity of dalchini or cinnamon is due to presence of Ascorbic acid and also by virtue of their hydrogen donating ability all of the tested compound and CBE exhibit reducing power, They were found to be potent in free radical scavenging activity especially against DPPH radicals, The peroxidation inhibiting activity of CBE recorded using an linoleic acid showed very good antioxidant activity.

Aim: To evaluate the antioxidant & Antibacterial activity of poly herbal formulation *Kalmi-dalchini*.

Objective
- To extract the chemical constituent from the *Kalmi-dalchini.*
- To investigate the phyto -chemical tests to the extract.
- To characterize the extract by spectral analysis like IR spectroscopy.
- To evaluate the compound for antibacterial and antioxidant activities by *in vitro* method.

Materials and Methods

The following experimental methods were used for the characterization of the extraction:

*Extraction of *Kalmi-dalchini* by soxhlet apparatus, using solvents like n-Butanol, methanol.

Apparatus

Beakers, spatula, water bath, soxhlet apparatus, water bath sand, Whatmann filter papers, china dishes, steam distillation apparatus, china dishes,steam distillation apparatus, cotton cloth,

Crude drug: Cinnamon bark

Chemicals: Methanol, n-Butanol, petroleumjelly powder (30gm+30gm).

Procedure for Extraction of Cinnamon Essential oil using Soxhlet Extraction method

[12]The extraction of cinnamon essential oil was carried out using soxhlet extraction method. An approximately 30gm of cinnamon powder was placed into the extraction thimble and covered with glass wool or Whatmann filter paper to prevent floating. The round bottom flask containing boiling chips was weighed. Then 250ml of methanol was poured into the round bottom flask, The soxhlet was heated at 68^0c at 1 atm pressure for 6hrs.The same method was applied to the other solvent which is n-Butanol. The heating temperature was changed to 117^0c for n-Butanol, Then both the extract were collected in the round bottom flask and kept for steam distillation separately, After distillation, pour into a china dish extract of cinnamon and other one was n-Butanol extract of cinnamon pour into another china dish extract of cinnamon and other one was n-Butanol extract of cinnamon pour into another china dish. It was kept for drying under room temperature.

Phytochemical test for Cinnamon Zeylanicum

Test for Alkaloids

2ml of each extract was treated with 2ml of wagner's reagent. A brownish red precipitate indicates the presence of alkaloids.

Test for cardiac glycosides

2ml of extract was treated with 2ml of chloroform and concentrated sulphuric acid was carefully added to form a layer. Deep reddish brown colour at the interface of steroid ring indicates the presence of Cardiac Glycosides.

Test for Flavonoids

2ml of extract was treated with 2ml of 10% lead acetate. Yellowish green colour indicates the presence of flavonoids.

Test for saponins

2ml of extract was dissolved in 2ml of Bendict's reagent. Blue black precipitate indicates the presence of saponins.

Test for Tannins

2ml of extract was treated with 0.1% ferric chloride. Brownish green indicates the presence of tannins.

Test for Terpenoids

2ml of extract was mixed with 2ml of chloroform and concentrated sulphuric acid was carefully added to form a layer, A reddish brown indicates the presence of Terpenoids.

Cinnamon aldehyde extract test (both methanol &n-Butanol extract)

Aldehyde test

2, 4 DNP test: Take 30mg of test sample (Both cinnamon methanol and n-Butanol extract in separate test tube in 2ml of 95%ethanol to that add 3ml of 2,4-dinitrophenylhydrazine reagent, shake vigourosly, precipitate forms immediately.

Tolle's test

Add test samples to 1ml freshly prepared Tollen's reagent, On gentle heating silver mirror or black precipitate was observed.

Chemical test

1or2 drops of ferric chloride was added to cinnamaldehyde samples (both cinnamon methanoland n-Butanol extract in separate test tube) produce brown colour.

All these tests confirmed both extracts of cinnamon have aldehyde functional group.

Cinnamon posses the Antibacterial and Antioxidant property for the determination proper activity some conditions are accomplished such as given below:

- There should be intimate contact between the test organisms and substance to be evaluated.
- Microorganism should be provided with the required condition of growth.
- Measurement of activity should be done correctly.
- Aseptic environment should be maintained.
- Condition should be maintained unchanged trough out the study.

Various methods with their own advantages and limitations have been used from time to evaluate he microbial activity of the drug. The antibacterial activity can be evaluated by the following techniques.

1. Agar streak dilution method.
2. Serial dilution method.
3. Agar diffusion method.

a) Cup.

c) Paper disc method.

4) Turbid metric method.

Study of Anti-bacterial activity

Strains

1. *Staphylococcus aureus* (Gram positive)
2. *Mycobacterium vareiance* (Gram negative)

Method

Agar paper disc diffusion method.

Dilution of the compound: 0.1,0.2,0.3,0.4,0.5ul/ml.

Composition of the media

Bacterial medium: Nutrient broth medium.

Peptone -5gm

Beef extract -3gm

Sodium chloride -5gm

Agar -20gm

Distilled water upto 1000ml

Preparation of standard as Ciprofloxacin dilution:

Ciprofloxacin tablet 150mg was collected and then caluclated and then caluclated their equivalent by following formula:

$$\text{Equivalent of tablet} = \frac{\text{Weight of drug to be taken}}{\text{Label claim weight}} \text{ x Average weight}$$

= 100/150x0.169
=0.112gm powder.

- 112gm ciprofloxacin tablet powder was weighed.
- Make upto 100ml with distilled water and then transfer into 100ml volumetric flask.

Preparation of stock solution of cinnamon Methanol &n-Butanol extract

- 10mg of cinnamon methanol extract was weighed and then make upto 100ml with ethanol as solvent then transfer into 100ml volumetric flask0.
- Similarly, 10mg of cinnamon n-Butanol extract was weighed and then make upto 100ml with ethanol as solvent then make upto 100ml volumetric flask.
- Both the solution mixed well.

Preparation of Cinnamon Methanol & n-Butanol extract

- The Cinnamon Methanol & n-Butanol extract dilution were prepared by using ethanol as solvent and by using serial concentration from 0.1-0.5ug/ml, and then dilute it with ethanol, Transfer these serial concentrations into five 10ml volumetric flask, Make upto 10ml with ethanol.

Procedure

Agar paper disc diffusion method

Antibacterial activities of cinnamon methanol & n-Butanol extract

- The *in vitro* antibacterial activities of the test samples (Cinnamon methanol & n-Butanol extract) were carried out by disc diffusion method, In the disc diffusion method, In the disc diffusion, nutrient agar was used as culture media and the discs were placed aseptically over the bacterial culture on nutrient agar plates are incubated at 37 ^{0}C for 24hrs after incubation for 24hrs.
- The zone of inhibition around the discs was measured by millimetre scale. These two bacteria were used *Staphylococcus aureus* and *Mycobacterium vareiance.*
- The diameter of zone of inhibition mean indicated by clear area which was devoid of growth of microbes was measured to determine antibacterial activity, Ciprofloxacin was used as Gram positive bacteria comparision of the antibacterial activity with the test samples which shows the zone of inhibition in these concentrations such as 0.2, 0.3, 0.4 and 0.5ug/ml, In gram negative bacteria, the ciprofloxacin in which the zone of inhibition was absent.
- The test samples (Cinnamon methonl & n-Butanol extract concentrations- 0.1, 0.2, 0.3, 0.4 and 0.5ug/ml) were compared with standard ciprofloxacin drug, but the test samples there zone of inhibition was absent in gram negative bacteria i.e,0.1u/ml.

Antioxidant studies by using hydrogen peroxide scavenging method

Materials

The materials used were hydrogen peroxide, phosphate buffer. The solvents and the other chemicals were of analytical grade.

Instruments

Absorbance was measured in UV-Visible spectrophotometer
P^H of buffer was measuerd in P^H meter.

Preparation of Phosphate buffer-P^H 7.4

Dissolve 2.38gm of disodium hydrogen phosphate, 0.19gm of potassium dihydrogen phosphate and 8.0gm of sodium chloride in sufficient water to produce 1000ml adjust the P^H if necessary.

Antioxidant activity by hydrogen peroxide scavenging method

A solution of hydrogen peroxide (40mm) was prepared in phosphate buffer (P^H7.3) of 3.4ml. Different concentrations (10, 20, 30, 40 and 50ug/ml) of synthesized compounds (ascorbic acid) were added to hydrogen peroxide solution (0.6ml, 40mm). Absorbance of hydrogen peroxide at 230nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. Hydrogen peroxide percentage scavenging activity was then caluclated using equation:

$$\text{Percentage inhibition}=\frac{\text{Absorbance of control-Absorbance of test}}{\text{Absorbance of control}}\text{x}100$$

Anti-bacterial activity

The extract was evaluated for antibacterial activity by using two Gram positive and Gram negative bacteria strains. It has been identified that the test sample Cinnamon methanol& n-Butanol extract show minute effect on bacteria. The test sample was compared with the standard ciprofloxacin. Ciprofloxacin drug shows the effect on bacteria, especially on Gram positive bacteria at the concentration such as 0.2, 0.3, 0.4, 0.5ug/ml.

Table 1: Standard drug

Ciprofloxacin Concentration ug/ml	Bacteria strains		Zone of inhibition (mm)
	Gram positive	Gram negative	
	Staphylococcus aureus	*Mycobacterium varince*	
0.1	+	+	No
0.2	_	+	0.1
0.3	_	+	0.2
0.4	_	+	0.3
0.5	_	+	0.5

Table 2: Cinnamon Methanol extract

Concentration ug/ml	Bacteria strains		Zone of inhibition (mm)
	Gram positive	Gram negative	
	Staphylococcus aureus	*Mycobacterium varience*	
0.1	+	+	No
0.2	+	+	No

0.3	+	+	No
0.4	+	+	No
0.5	+	+	No

(+) =presence of growth, (-) =Absence of growth No = No zone of inhibition

Table 3: Cinnamon n-Butanol extract

Concentration ug/ml	Bacteria strains		Zone of inhibition(mm)
	Gram positive	Gram negative	
	Staphylococcus Aureus	*Mycobacterium Varience*	
0.1	+	+	No
0.2	+	+	No
0.3	+	+	No
0.4	+	+	No
0.5	+	+	No

(+) = Presence of growth, (-) =Absenc of growth No =No zone of inhibition

Antioxidant activity

The extract of cinnamon was evaluated for antioxidant activity using *in vitro* hydrogen peroxide scavenging method and ascorbic acid is used as standard. The extract at lower concentration had shown greater activity compared to the standard.

Table 4: Absorbance of standard (Ascorbic acid): 3.057

Concentration (ug/ml)	Methanol extract	Butanol extract
0.1	0.846	0.744
0.2	1.020	o.933
0.3	1.260	1.162
0.4	1.592	1.304
0.5	1.763	1.591
Standard		

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