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IDENTIFICATION OF PHYTOCHEMICAL, ANTIBACTERIAL, ANTIOXIDANT PROPERTY OF CINNAMON AND FENUGREEK

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INTRODUCTION:

Spices played an important role in the history of exploration and development, are no longer luxury items of guat cost.

They are widely used by the meat, sauce, canning, Frozen food industries, and food manufacturing industry generally. They are also used in the cosmetic and perfumery industries, including its use in soap and toothpaste. The essential oil of cinnamomum are of some importance in the preparation of liqueurs and cordial. They are also used in various ayurvedic and allopathic medicine.

Bakers use it liberally in cookies and in hot drinks. Cinnamomum are said to be among the oldest spices. Cinnamomum are said to be among the oldest spices. Cinnamomum has fragrant, sweet and warm taste.

Commercial essential oil production industry used several aromatic plant species for of all extracting high quality essential oil.

Cinnamon is a highly valued spice whose bark is widely used as a spice. It is mainly used in cookery as a spice and by various industries for foodstuff, flavouring f or fragrance and essence perfumes, and medicinal products.

Cinnamomum stands out of all spices in its "warmth" and ranks as second to pepper.

It is indigenous in Sri lanka, which still produces the largest quantity and best quality, mainly in the form of quills.

Cultivation of cinnamomum:

Around 27,000-35,000 annual tons cinnamon is globally produced. It is mostly raised in China, Seychells, Madagascar and Srilanka, additionally its cultivated on a little scale in Vietnam and India.

It's a hardy plant in terms of its suitableness for its cultivation in various weather conditions.

The optimal temperature for the cultivation of cinnamon ranges between 20-30°C, with a yearly rainfall ranging between 1250-2500mm. Cinnamon is usually propagated by dried seeds and vegetative propagation.

TYPES OF CINNAMON:

There are mainly four types of cinnamon:

True cinnamon or ceylon cinnamon or Mexican cinnamon (cinnamomum zeylanicum)

Indonesian cinnamon (cinnamomum burmanni)

Vietnamese cinnamon (cinnamomum loureiroi)

Cassia cinnamon or Chinese cinnamon (cinnamomum aromaticum)

CINNAMON AND HEART DISEASE:

An animal study on Sprague Dawley rats evaluated the effect of c.cassia on Ischemic heart disease. The active components cinnamaldehyde and cinnamic acid are said to be cardio protective due to their ability to produce nitric oxide as well as the associated ant-inflammatory property.

Its vasorelaxation effect has also been attributed the cinnamaldehyde component which inhibits the L type calcium channels.

FENUGREEK:

Fenugreek belongs to fabaceae family, it was named, Trigonella, from latin language that means "little triangle" due to its yellowish-white triangular flowers. Its is named as Methi (Hindu, Urdu, Punjabi, and Marati), Hulba (Arabic), Moshoseitaro (Greekk), Uluva(Malayalam) heyseed in English.

Fenugreek (Trigonella Foenum-graecum L.) is one of the oldest medicinal plants from Fabaceae family originated in central Asia ˜4000 BC.

Fenugreek seeds contains a substantial amount of fiber, phospholipid, glycolipids, oleic acid, Linoleic acid, choline, vitamin A, B1, B2, C, nicotinic acid, niacin, and many other functional elements. Despite its exceptional

nutritional and medicinal values, only a few studies have been done for its genetic enhancements and development of production agronomy.

In this review, we have discussed the morphology, adaptability, nutritional constituents and associated functionality and medicinal significance of fenugreek, its ethno-historical uses, pharmacological assumption have also been discussed.

Legume plants, including fenugreek (trigonella foenum-graecum L.), constitute high quality foods that deliver nutritional and functional advantages at a low price.

Fenugreek is frown mainly in china, India, Turkey, Canada , Australia, Northern and southern, Africa and southern Europe.

SCIENTIFIC CLASSIFICATION:

KINGDOM : plantae
DIVISION : mahnoliophyta
CLASS : mahnoliopsidaa
ORDER : fabales
FAMILY : fabaceae
GENUS : trigonella
SPECIES : foenum-graecum
BINOMIAL NAME: trigonella foenum-graecum.

MORPHOLOGY OF SEED:

APPEARANCE: Solid rhomboidal seeds, 3-5 mm long , 2 mm thick, Hard, pebble-like .
COLOUR: Yellowish brown or light brown.
ODOUR: Spicy
TASTE: Bitter.

PHYTOCHEMICAL ANALYSIS:

Guevarra.B et,al (2005), " A guide book to phytochemical screening: phytochemical and biological manila: UST Publishing House"

ANTIMICROBIAL ACTION:

CINNAMON: Hameed I, Altameme H, Mohammed G. Evaluation of Antifungaland Antibacterial Activity and Analysis of Bioactive Phytochemical compounds of Cinnamomum Zeylanicum (Cinnamon Bark) using Agar diffusion method. Oriental Journal of chemistry (Internet). Oriental scientific Publisihing Company; 2016 Aug 23; 32(4):1769-88. Available from:http://dx.doi.org/10.13005/ojc/320406 [Phytochemical screening and Antimicrobial Activity of "Cinnamon zeylanicum"]

FENUGREEK: Premanath R, Sudisha J, Lakshmi Devi N, Aradhya SM (2011) Antibacterial and antioxidant activity of Fenugreek (*Trigonellafoenumgraecum* L.) leaves. Res J Med Plants 5: 695-705.

ANTIOXIDANT PROPERTY:

DPPH (2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl) Test

CINNAMON: Nakatani,N. In: phenolic compounds in food and their effects on health (Huang, M. & Lee, C.ed). Am. Chem. Soc., Washington,.p. 39-48 1992.

FENUGREEK: C. Billaud, Sciences-des-ailments., 21 (2001) 3.

Y. Sauvaire, G.Ribes, J. C. Baccou and M. M. Loubatieres-Mariani, Lipids Mar., 26 (1991)191.

Shahidi, F. In: Natural Antioxidant (SHAHIDI, F.ed). AOCS Press, Champaign, p. 1-11 1997.

ESTIMATION OF TOTAL PHENOLS:

Total phenolic compound was determined by Foli-ciocalteau reagent method (Kaur C, Kapoor HC., 2002). 2ml Ethanol Acetone and Aqueous extract of cinnamon and fenugreek was taken. Then, add 1ml of Folin ciocalteau reagent

(1:10 diluted with distilled water). Then, 1ml of 20% (W/V) Na2CO3 solution was added and shaken well. The mixture was then allowed to stand for 30 min incubation in dark at room temperature. The absorbance was measured at 765 nm. The total phenolic content was expressed in terms of gallic acid equivalent (µg/mg of extract).

ESTIMATION OF TOTAL FLAVONOIDS:

The total flavonoid content of cinnamon and fenugreek extract was determined using aluminium chloride reagent method with slight modifications (Ordonez AAL, Gomez JD, Vattuone MA, IsLa MI, 2006). 2ml Ethanol, Acetone, and Aqueous extract of sample was taken. Then add 0.5mL of 5% (W/V) sodium nitrite solution. Then, 0.5 mL 10% (W/V) aluminium chloride solution was added followed by 50 µL of 1 M NaOH solution was expressed as querfetin equivalent (µg/mg of extract).

Estimation of Carbohydrate by Anthrone method:

Total carbohydrate was estimate by anthrone method (Biochemistry Den, 2021). 1ml of ethanol, aqueous and acetone extract of cinnamon and fenugreek was taken and add 4ml of anthrone reagent and mix well, water bath for 10 min then cool the test tube in room temperature. Measure the optical density in a phototelectric colorimeter at 620nm.

Estimation of Protein by Lowry's method:

Total protein content was estimated using Lowry's method (Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall,RJ.). The reagent prepared were solution A (2% sodium carbonate in 0.1N NaOH) and solution B (0.5% copper sulphate solution in 1% sodium potassium tartarate). Alkaline copper sulphate solution was prepared by missing solution A and B in the ration of 50:1. Folin-Ciocalteau reagent was diluted with equal volume of water just before ue. 5ml of copper sulphate solution was added to each extract and incubated at room temperature for 10 min followed by addition of 0.5ml FC reagent. Absorbance was recorded at 660nm. BSA was taken as standard.

Result:

Phytochemical qualitative analysis:

Bioactive compounds	Ethanol	Aqueous	Acetone
Alkaloids	+	+	+
Steroids	+	+	+
Terpenoids	+	+	-
Flavonoids	+	-	+
Phenol	+	-	+
Tannins	+	-	+
Saponins	-	+	+
Glycosides	+	-	-
Phlobotannins	-	+	-

Table no:1 shows the phytochemical analysis of bioactive compounds. Wagner's test for the alkaloids showed the presence of reddish brown precipitate. Alkaline test for flavanoids was negative.

Benedict's test for the test of reducing sugar showed the presence of green or yellow or red colour. The ninhydrin tests for amino acids was negative.

Salkowski's test for steroid showed red colour in the lower layer of the extract. Blue colour solution observed in the tests for phenols and tannins by Ferric chloride test.

Tests for terpinoids , Salkowski's test showed the presence of yellow colour at the bottom. Negative results was obtained in Biuret test for proteins.

In Froth test for saponins observed foam formation except in ethanol extracts.

Anti-microbial Activity of Cinnamon:

Table 2: Zone of Inhibition of Cinnamon Extract against *Escherichia coli* (gram-negative)

Dilution of cinnamon extract(µl/ml)
Zone of Inhibition (mm)

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Cinnamon extract	**Concentration of extract**		
	30	40	50
Control	-	-	-
Ethanol	7 ± 2	7 ± 4	7± 6
Aqueous	-	-	6 ± 2
Acetone	10± 3	10± 5	10 ± 7

Table 3: Zone of Inhibition of Cinnamon Extract against *Enterococcus faecalis* (gram-positive)

Dilution of cinnamon extract(µl/ml) **Zone of Inhibition (mm)**			
Cinnamon extract	**Concentration of extract**		
	30	40	50
Control	-	-	-
Ethanol	7 ± 4	7± 5	7± 6
Aqueous	6 ± 3	6 ± 4	6 ± 7
Acetone	11 ± 5	11 ± 8	11 ± 10

Table 4: Zone of Inhibition of Cinnamon Extract against *Salmonella typhi* (gram-negative):

Dilution of cinnamon extract(µl/ml) **Zone of Inhibition (mm)**			
Cinnamon extract	**Concentration of extract**		
	30	40	50
Control	-	-	-
Ethanol	14 ± 9	14 ± 10	14± 13
Aqueous	8 ± 3	8 ± 4	8 ± 6
Acetone	15±8	15±10	15± 11

Anti-microbial activity of Fenugreek:

	Zone of inhibition (mm)		
Extract	E. coli	Staphylococcus	**Salmonella typhi**
Ethanol	-	-	-
Aqueous	**5**	**5**	**5**
Acetone	-	-	-

Anti-microbial Activity

The results of the agar diffusion test (Cork borer method) revealed that the various extracts of cinnamon showed different degrees of growth inhibition, depending upon the bacterial strains. The acetone extract of cinnamon showed notable antibacterial activity against gram-positive bacteria and gram-negative bacteria. It is well known that most spices are more active against gram-positive bacteria than gram-negative bacteria. This study showed that acetone and ethanol extracts of cinnamon were more effective against gram-positive bacteria in vitro. The sequence of antibacterial activity against *E. coli* is as follows with their zone of inhibitions:

Acetone extract (10) > ethanol extract (7) > Aqueous extract (6)

The sequence of antibacterial activity against *Enterococcus faecalis is* as follows with their zone of inhibitions

Acetone extract (11)> ethanol extract (7)> Aqueous extract (6)

The sequence of antibacterial activity against *Salmonella typi is* as follows with their zone of inhibitions.

Acetone extract (15)> ethanol extract (14)> Aqueous extract (8)

The sequence of antibacterial activity against *Escherichia coliis* as follows with their zone of inhibitions.

Estimation of total phenol and falvonoid :

Sample	Total phenolic content (mg GAE/100g)	Total Flavonoid content (mg of RE/g)
Cinnamon	18.94 ± 0.46a	41.92
Fenugreek	150.80±0.33	72.70±0.04

Estimation of Antioxidant properties:

Sample	DPPH radical scavenging activity based on IC50 value (mg/ml)	Total antioxidant capacity (mg GAE/g)
Cinnamon	0.009±0.76	149.15 ± 1.73a
Fenugreek	51.6±0.07	78.0±0.02

DISCUSSION

Spices have played an important part in the lifestyle of people in certain areas of the world since ancient times. They have played many roles in the history of dyeing agents, aromas, preservatives, food additives and medicines. The molecular basis for these actions was the active phytochemicals derived
from these spices. Cinnamon is a commonly used cooking spice with potentially medicinal effects. Cinnamon is medicine for respiratory and digestive conditions in native ayurvedic medicine.

In this study, cinnamon bark has been analyzed using standard methods against pathogenic bacteria for the phytochemical and antibacterial evaluation. The phytochemical screening included qualitative chemistry and primary and secondary metabolite determination tests. It has a variety of active plant chemicals that attributes the medicinal properties of this plant. There is a global concern about the emergence of resistant bacterial and fungal strains due to the overuse of antibiotics.

The anti-microbial activity of Cinnamomum has been tested. Ethanol, Acetone, and water were used to produce cinnamon extracts. Table No. 5,6,7 shows the antimicrobial properties of the extracts.

In order to find out the cinnamon-based secondary metabolites that govern its antimicrobial properties, the extracts were phytochemically analyzed. Phytochemicals are an essential component of our diet, that is, secondary metabolites of plants. Figure 2, 3, and 4 shows the zone of inhibition of extracts
against *Escherichia coli*, *Enterococcus faecalis* and *Salmonella typhi* respectively.

With antioxidant properties imparted by flavonoids and tannins, saponins contain anti-cancerous and lower levels of cholesterol. As anti-malarial compounds and as analgesics, alkaloids are used.

The results of the phytochemical analysis found the Acetone and Ethanol extract of cinnamon bark containing all six tested plant chemical products. Different biochemical tests characterized the isolates and were susceptible to cinnamon extracts. Cinnamon chloroform extracts, cinnamon methanol extract, cinnamon aqueous extracts with a minimum inhibitory concentration of 50mg / ml were the order of bactericidal activity.

The anti-microbial activity was performed to determine the microbial activity of cinnamon by observing the zone of inhibition and thus the anti-microbial activity of the cinnamon extract was determined.

For medicinal purposes, $1/5^{th}$ of all plants found in India are used. Due to its distinct strong smell of different compounds, the bark of cinnamon is widely used as a spice. Research and experimentation have been carried out on the spice's phytochemistry and antimicrobial properties.

The presence of certain phytochemicals in the extracts has been certified for antibacterial activity. Studies suggested that Cinnamomum antibacterial activity might be different because of its major component cinnamaldehyde, and its properties.

In cinnamon the cinnamaldehyde and eugenol plays the main bioactive compound, which acts against different microbial strains. And bioactive compound extracted in Acetone and Ethanol is more than aqueous, ethyl
acetate.