PHARMACOGNOSTIC PROFILE, PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY ESTIMATION OF BARK EXTRACTS OF *CINNAMONUM ZEYLANICUM*

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Background: There are several cinnamon species that are cultivated worldwide. Since ancient times, cinnamon bark has been utilized as a traditional remedy. It is imperative to provide scientific evidence for the existence of the medicinally effective active ingredients in *Cinnamomum zeylanicum*.
Aim: The goal of the current study was to assess the pharmacognostic profile, phytochemical composition and antioxidant activity of *C. zeylanicum* bark.
Materials and Methods: The methanolic extract of *C. zeylanicum* was quantitatively evaluated for total phenol content, total flavonoid content, and total saponin content after preliminary pharmacognostic screening of several extracts (methanol, ethanol, acetone, n-hexane, and water). By using the ferric reducing antioxidant power (FRAP) test, the antioxidant property was assessed.
Results and Discussion: Different phytochemical concentrations were discovered in the *C. zeylanicum* species. The methanolic extract had the greatest overall polyphenol concentration of the five components. The total phenol content was 436.66 mg/g GAE and the total flavonoid content was 41.92 mg/g rutin. Amount of saponin discovered: 81.05 mg. According to the FRAP method's estimate of antioxidant potential, the highest ferric reducing antioxidant power is 1.377 at a concentration of 1000 g.
Conclusion: As a result, the research established that C. zeylanicum has a number of active components and the ability to serve as an antioxidant, allowing it to be used regularly to treat and prevent illness.

Keywords: Antioxidant, ferric reducing antioxidant power, photochemical, thin layer chromatography, fluorescence.

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1. Introduction

For thousands of years, natural remedies found in nature have been the finest source of traditional medicine (Cragg *et al.*, 2001). Since ancient times, medicinal plants have been thoroughly studied. In the literature of ayurveda, allopathy, and homeopathy, there is no discernible difference in the values of medicinal herbs or in treatment methods (Ribeiro De Pavia *et al.*, 1995). Phytochemicals are compounds produced by plants in the truest sense. The main phyto-components in the plant that give it its usefulness include, flavonoids, alkaloids, saponins, coumarins, phenol carboxylic acids, and terpenes. These plants have particular traits and qualities. As a result, it would be possible to ascertain many biological activities of plants through the analysis of these constituents in plants (Rajula and Ujwala 2010; Harborne 1973). According to the World Health Organization (WHO), traditional plants provide primary healthcare to over 80% of the world's population (Gupta *et al.*, 2013).

Using herbal plants to depict human interactions with the environment has a long tradition in Asia. Both infectious and chronic diseases are treated with these various natural substances. (Ravi *et al.*, 2015; Girach *et al.*, 1994). Since many centuries, almost the entire population of India has used traditional medicine. It is thought that medicinal plants contain novel chemicals with potential therapeutic benefits (Edeoga *et al.*, 2005).

Numerous plants have significant concentrations of phenolic antioxidants.In addition to having analgesic properties, phytochemicals have anticancer, antiatherogenic, antiulcer, antithrombotic, anti-inflammatory, immune-modulating, antimicrobial, and anti-inflammatory properties. Natural antioxidants, primarily those derived from plants, are now much more widely used (Rao *et al.*, 2014; Yen *et al.*, 2002). The most prevalent and widely dispersed class of plant phenolics is flavonoids (Harborn *et al., 1998*).

Free radicals (ROS / RNS) can harm biomolecules when they are present in high concentrations because they cause oxidative and nitrosative stress. Since free radicals have unpaired electrons, they react toform bonds with electron pairs. DNA mutations, membrane protein damage, and membrane disintegration are all results of free radical oxidation (Ravishankar *et al.*, 2007). Excess ROS can damage biomolecules such as lipids, proteins, and DNA, resulting in oxidative stress, which is a role in diabetes, neurological diseases, rheumatoid arthritis, cataracts, cardiovascular illnesses, respiratory diseases, and ageing (Rates 2001). Antioxidants are molecules that can stop free radicals from oxidizing. Antioxidants help free radicals by giving up their electrons, which stabilizes the atoms or molecules of free radicals and stops them from harming healthy cells. Antioxidants are naturally formed in our bodies to control the reactions of free radicals (Priyanka 2013; Auwal *et al.*, 2014). However, due to their scarcity and inability to effectively combat glaring free radicals, external antioxidants are required (Majid *et al.*, 2021).

Cinnamon (Cinnamomum burmannii), which can be found in nature, is one source of antioxidants. There are numerous species and cultivars of the native plant known as cinnamon, which is found in Southeast Asia, China, and Australia. In Indonesia, Sumatra and Java are where you can find cinnamon. The bark and branches of cinnamon, an export from Indonesia, can be used as seasonings. Cinnamon

contains phytochemicals in its bark, branches, twigs, and leaves. In its natural or powdered state, as well as in the form of an essential oil or oleoresin, cinnamon bark can be used right away. The cinnamon tree's bark, branches, twigs, and leaves can all be utilized to create oil (WHO, 1998)

For many years, cinnamon has been a popular spice used in dishes from all over the world (Rao & Gan, 2014). Cinnamomum cassia (L.) and Cinnamomum verum J. Presl are among the first known spices used by humans, J. Presl barks were sought for by the majority of European travelers in the 15th and 16th centuries4 (Avula *et al.*, 2015).

Figure 1 Cinnamon whole plant and bark.

Since ancient literature from 4,000 years ago has been preserved, its therapeutic and gastronomic properties have been thoroughly documented (Rao & Gan, 2014). In most regions of the world, essential oils and plant parts are the source of spices and flavoring compounds. In order to preserve food products and enhance their pharmacological effects, it is also employed as a condiment in spices, sauces, bread, confectionery, and beverages (Pittman, 2011). It was also used to embalm mummies in the Egyptian and Roman empires due to its aroma and flavoring qualities (Ribeiro-Santos *et al.*, 2017). According to Li and Parsons, 2015, cinnamonaldehyde, the major active component of cinnamon, is responsible for adding taste, odour, and flavour to foods. It also provides resistance to oxidative stress, microbial infection, and other chronic disorders. (Li and Parsons, 2015).

2. Materials and Methods

Specimens (bark) of *cinnamon zeylanicum* was collected from local marketof Nadaun, Distt Hamirpur (H.P.). Intially physicochemical examination was carried for parameters such as LOD, swelling index, ash value (total ash, acid-insoluble ash, and water-soluble ash), and pH (1% and 10% solution) as per standard procedures (WHO, 1998). The bark was then subjected to macroscopic examination and for preliminary phytochemical studies the bark extracts (ethanol, methanol, acetone, n-hexane and aqueous extract) were tested separately for the presence of active constituents such as alkaloids, flavonoids, saponins, tannin, steroid,sterols, proteins, carbohydrates, glycosides and total phenol (Trease and Evans, 2005). The quantitative analysis of total phenolic content (Standard-Gallic acid) (Djeridane *et al.*, 2006), total favonoid content (Standard- Rutin) (Zhishen, *et al.*, 1999), and total saponin content was performed using spectroscopic techniques.

The TLC of the extracts was done using preparative TLC plates using methanol: DCM (1:9) as Mobile

phase. The TLC was analyzed under UV and 2,4-DNP was used as a spraying agent (Sharma *et al.,* 2011). Daylight and UV light at 254 nm and 366 nm were used to investigate the fluorescence properties of powdered bark for color changes (Majid *et al.,* 2021). Finally Ferric reducing antioxidant power was determined by FRAP method as per procedure (Zhong and Shahidi, 2015).

3. Results and Discussions

The existence of various phytochemicals with pharmacological and commercial uses, including fragrances and flavor, colors, gums/resins, and fiber, with a significant impact on the health and business sectors, was discovered by phytochemical screening of cinnamon extracts. The bulk of significant secondary metabolites are generated by the plant in negligible concentrations. Table 1& 2 displays the physicochemical analysis and macroscopic examination results of cinnamon bark respectively. Table 3 displays the results of the qualitative phytochemical assay. Preliminary phytochemical analysis of the bark extract from C. zeylanicum found alkaloids, flavonoids, terpenes, sterols, anthocyanins, tannins, , proteins, glycosides, polyphenols, carbohydrates and saponins in the current study. Phenyl propanoids, terpenes, flavonoids, and saponins of the C. zeylanicum species are water and oil soluble. The methyl hydroxychalcone polymer is created when all of these components are combined, and it is thought to be the main polymer responsible for reduction in blood glucose levels in people with type 2 diabetes (Khunoana *et al.,* 2012). In their study, Saleem *et al.,* (2010) found that the bark of C. zeylanicum contains bioactive substances such polyphenols, flavonoids, saponins, tannins, and coumarins. The plant C. zeylanicum was used in the current investigation, and ethanolic and methanolic extracts of the plant were used. A wide variety of phytochemicals, including flavonoids and polyphenols, were found. The dried crude powder of C. zeylanicum bark was utilized for the quantitative evaluation of TPC, TFC, and TSC after being examined for the presence of total phenols, alkaloids, flavonoids, tannins, terpenes, sterols and saponins. Due to the high phenolic and flavonoid content of cinnamon, it possesses exceptional antioxidant effects. The outcomes were represented by the variation in absorbance at 765 nm between the test sample and the reference gallic acid solution. Following total saponin content, which was around 81.05 mg (Fig 3), the total phenol content (Fig 4) and total flavonoid content (Fig 5) of bark extract were determined to be 436.66 mg/g gallic acid equivalent (GAE) and 41.92 mg/g rutin, respectively.

The ferric reducing antioxidant test assesses an antioxidant compound's ability to donate electrons. The production of a colourful ferrous complex arises from the reduction of ferric to ferrous ion. Figure 6 depict the FRAP test of C. zeylanicum. The most recent research is in line with earlier studies (Jasper *et al.,* 1958), which discovered that secondary metabolites (phytochemicals) are in charge of the cinnamon bark's antioxidant activity, making it an important therapeutic agent. The outcomes were expressed by contrasting the test sample's change in absorbance at 700 nm with that of the L ascorbic acid reference solution. The Fe 3 + reduction activity data suggested that C. zeylanicum has the potential to reduce. The maximum ferric reducing antioxidant power was measured at 1000 g and was found to be 1.367. Advanced glycation end products, which cause problems for diabetics, were prevented from developing by extracts from cinnamon bark. This inhibition is thought to be brought on by the extracts' ability to

trap reactive carbonyl species in phenolic compounds. In those who received cinnamon extract, the antioxidant variable ferric reducing antioxidant capacity increased (Varalakshmi *et al.*, 2014). Studies on the antioxidant properties of C. zeylanicum bark revealed improved free radical scavenging abilities. According to Elmastas et al. (2006), a number of mechanisms have been proposed to explain antioxidant activity, including the blockade of chain initiation, the binding of ion catalysts, the dissolution of peroxides, and the inhibition of further hydrogen removal. According to a study by Roussel et al. (2009), adding water-soluble C. zeylanicum compounds to the diet as an antioxidant may lower risk factors for diabetes and cardiovascular disease.

Table 1: Physicochemical examination of *Cinnamomum zeylenicum*

S.No.	Parameters	Value (Mean± S.E.M)
1	LOD	10.30 ± 0.30
2	Swelling index%	43% ± 3
3	Water soluble ash	3.22 ± 0.80
4	Acid insoluble ash	3.63 ± 0.67
5	Total ash	10.70 ± 0.77
6	pH (1% solution)	6
7	pH (10% solution)	6

Table 2: Macroscopic examination of bark of *Cinnamomum zeylenicum*

S.No.	Macroscopic characteristics	Observation
1	Shape	Bundles and wrapped in Coir
2	Size	1m long, 0.5 mm in thickness with a 6-9 mm diameter
3	Color	Dark yellowish brown
4	Odour	Aromatic
5	Taste	Sweet

Figure 2 Different types of cinnamon extract

Table 3: Phytochemical Screening of Different Extracts of *Cinnamomum Zeylenicum*

Phytoconstituents	Ethanol	Methanol	Acetone	Water	Petroleum ether
Alkaloid examination					
• **Wagner test**	+	-	-	+	-
• **Mayer test**	+	-	-	+	-
Flavonoid examination					
• **NaCl test**	-	-	-	-	-
• **Lead acetate test**	-	-	-	-	-
Saponin examination					
• **Foam test**	-	-	-	-	-
• **Liberman test**	+	+	+	+	-
Tannin examination					
• **Ferric chloride test**	+	+	+	+	+
Steroid/ terpenoid examination	+	+	+	+	+
• **Chloroform test**					
Sterol examination					
• **Liberman-Burchard test**	+	+	+	-	-
Protein examination					
• **Biuret test**	-	-	-	-	-
• **Million tests**	-	-	-	-	-
Carbohydrates					
• **Molisch test**	-	-	-	-	-
Glycosides					
• **Killer killiani test**	-	+	-	-	+
Total phenol					
• **Ferric chloride test**	-	-	-	-	-

Table 4: Quantitative Analysis of Methanolic Bark Extract of *Cinnamomum Zeylenicum*

S.No.	Phytochemicals	Amount (mg/g)
1	TPC	436.66
2	TFC	49.92
3	TSC	81.05

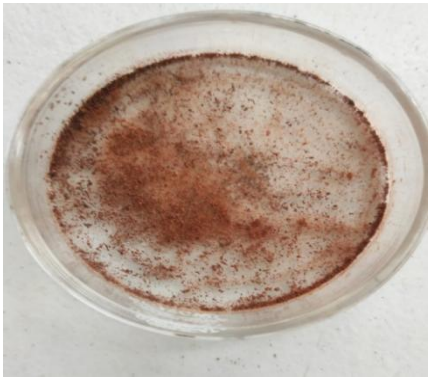

Figure 3 Total saponin content

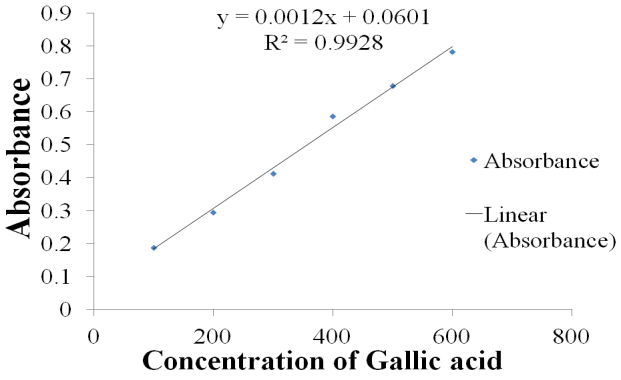

Figure 4 Total phenolic content

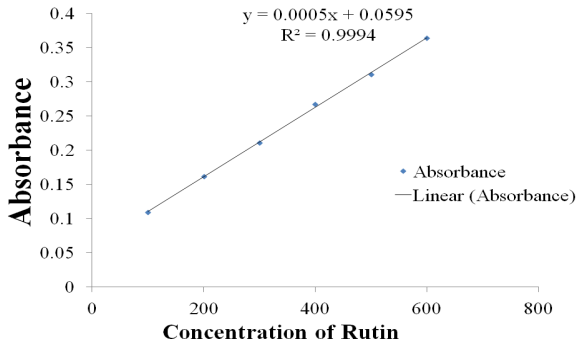

Figure 5 Total flavonoid content

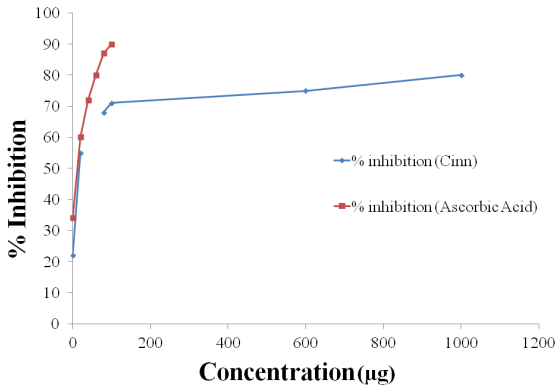

Figure 6 FRAP of cinnamon and ascorbic acid

Table 5: Fluorescence Analysis of Powdered Bark of *Cinnamomum Zeylenicum.*

S.NO	Treatment	Day light	Uv light at 254 nm	UV light at 365 nm
1.	PC + 5% Ferric Chloride	Yellowish brown	Green	Dark brown
2.	PC + 1%-Iodine solution	Light green	Dark green	Black
3.	Powder + Benedict's reagent	Brown	Dark green	Dark brown
4.	Powder + 10%-Potassium Dichromate solution	Dark yellow	Florescent green	Reddish brown
5.	PC+ NaOH Solution (1N)	Brown	Dark green	Dark brown
6	PC + Conc. H_2SO_4	Reddish brown	Black	Black
7	PC + Ethanol	Yellowish brown	Light green	Reddish brown
8	PC +Methanol	Brown	Dark green	Dark brown
9	PC + Picric acid	Yellowish-brown	Green	Dark green
10	PC + Acetic acid	Light brown	Green	Black
11	PC + Conc.HNO_3	Yellow	Light green	Dark green
12	Powder + Hexane	Brown	Reddish brown	Dark brown
13	PC + Conc. HCl	Brown	Light green	Brownish-black
14	PC + $CHCl_3$	Yellow	Light green	Brown
15	PC + Liq.NH_3	Reddish brown	Greenish yellow	Dark brown
16	PC + Conc. HNO_3	Yellowish brown	Light green	Brownish-black
17	Powder +Acetone	Light yellow	Light green	Light brown
18	PC + Distilled H_2O	Brown	Light green	Black
19	Powdered cinnamon (PC) alone	Brown	Green	Brown-black

Table 6: TLC of different extracts of *Cinnamomum zeylenicum*

S.no.	Extract	Mobile phase	Number of spots	Rf Value	Visualizing technique
1.	Ethanol Extract	Methanol: DCM	1	0.7	UV- chamber & 2,4-DNP
2.	Methanol Extract	Methanol: DCM	1	0.7	UV- chamber & 2,4-DNP
3.	Acetone Extract	Methanol: DCM	1	0.7	UV- chamber & 2,4-DNP
4.	n-Hexane Extract	Methanol: DCM	1	0.7	UV- chamber & 2,4-DNP
5.	Distilled water Extract	Methanol: DCM	0	0.7	UV- chamber & 2,4-DNP

Conclusion

The phenols, flavonoids, and alkaloids in the ethanolic and methanolic bark extracts were found to be in significant concentration in the quantitative assays, indicating that they may be essential bioactive substances. *Cinnamum zeylenicum* ethanolic, methanolic and acetone extracts had more antioxidant scavenging activity than aqueous and n-Hexane extracts, which had very little activity. Thin Layer Chromatography and spraying reagent 2, 4-DNP were used to separate and identify the principal active components. As a result, cinnamon has the potential to be a great source of natural antioxidants that are crucial for treating and preventing oxidative diseases.

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