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PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY OF CINNAMOMUM *TAMALA* LEAF EXTRACT

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Abstract: *Cinnamon tamala*, is a tree that is also known as bay leaf or *tejpatta*, it is native to India and included in the family Lauraceae. It has been proved that this plant has various pharmacological activities such as anti-tumor, anti-inflammatory, anti-oxidant, etc. In this present study, the phytochemical profile of the leaf is investigated both qualitatively and quantitatively. After the phytochemical investigation of the leaf, antioxidant potency for free radical scavenging (ABTS and DPPH) was examined. The presence of various alkaloids, steroids, and flavones was revealed by qualitative assessment. Cinnamon oil exhibited highest antioxidant activity having IC_{50} value of 40.85± 4.96 and 18.57 ± 0.10µg/ml in DPPH and ABTS assay.

Key words: C. *tamala*, antioxidants, ABTS method, Tannin**,** saponins , glycosides

1.Introduction: Medicinal plants are those plants that are used for the treatment, mitigation, or prevention of diseases[1,2]. Some drugs are derived from different plant parts like leaf, stem, bark, etc. and these drugs are known as herbal drugs[3]. Herbal drugs can be available at low cost. In India, there is wide use of plants for folk medicines and also in the pharmaceutical industry. In the native system of medicines like Siddha, Unani, and Ayurveda, there is the use of folk medicine. 4.5 million plant species were estimated in India, but still some plant species were explored for pharmacological and photochemical purposes[4]. In the pharmacological industry, secondary metabolites (extracts) are used for novel preparation of drugs[5]. According to a WHO survey, around 80% population of India relies on the use of herbs as primary medicine because

herbs are cost-effective with lesser side effects. Commercially herbal medicines are formulated on a wide range because of above said benefits[6].

In traditional medicines medicinal plants and herbs play a vital role. Plants and natural products play a significant role in human healthcare. The system of traditional medicines is revived day by day because of curative effects, ease of availability, healing inherently, and lesser side effects[7,8].

Nature is diversified with various medicinal plant species, and Cinnamomum species is one of them. This genus Cinnamomum constituted around 350 species worldwide. these species are native to Australia, Asia, and the Pacific Islands and rainy forests are required for growth at altitudes, which are varying[5,9].

C. tamala is included in the Lauraceae, the other name is Indian Cassia and the leaf is called bay leaf. It is a tree with approx. 8m in height and can be cultivated. Tropical and sub-tropical Himalayas at altitudes 900-2500m are the natural habitat for the growth of this tree. The taste of this plant resembles clove and the odor is like pepper. Generally, this is used as a spice but some pharmacological activities like anti-diabetic, gastro-protective, anti-oxidants, anti-inflammatory, and free radical scavenging activity are also revealed by some studies. Oxidative damage is reduced by the extract of plant and it is also useful in renal toxicity. It is also useful in the treatment of numerous diseases like rectal, anal diseases, cancer, flatulence, liver-spleen diseases etc[10].

2. Material and Method

2.1Chemicals and reagents

Methanol, HCl, ethanol, acetic acid, gallic acid, acetic acid(glacial), n-hexane, sulphuric acid, sodium nitrite, sodium hydroxide, aluminum chloride, ferric chloride, quercitin.. Ferric chloride hexahydrate, DPPH, sodium acetate, sodium carbonate, vitamin C, mercuric chloride,Folin-Ciocalteu phenol reagent, iodine, potassium acetate, sodium carbonate, potassium iodide, aluminum trichloride, Dragendorff ’reagent were purchased from CDH, Delhi. Methanol, ethanol, n-hexane, and chloroform were purchased from Merck. Analytical grade chemicals are used.

2.2 Collection and identification of plants

Fresh *Cinnamomum tamala* leaves were collected from the region of Behrampur, West Bengal *C. tamala* leaves were identified from the National Institute Of Science Communication And Information Resources, New Delhi with letter reference no NISCAIR/RHMD/Consult/2016/2983-10, dated 23/09/2016.

2.2.1 Preparation of extracts

After being sun-dried, the leaves of C. *tamala* were broken down into tiny pieces. 500 g of dried and powdered plant material was extracted sequentially in a Soxhlet device using n-hexane, chloroform, and methanol as the solvents with increasing polarity. The plant material was boiled in distilled water for two hours on a hot plate to create the aqueous extract.The extracts were collected and kept in a desiccator under vacuum(6).

2.2.2Isolation of oil

C. tamala leaves were cut into small pieces and oil was isolated via hydro distillation method using Clevenger apparatus[12].

3.Phytochemical Screening

The presence of phytochemicals such flavonoids, polyphenols, flavones, terpenoids, and carbohydrates was further investigated in the crude extract. The assessment was conducted using accepted techniques. This assessment is based on the color change and formation of precipitates[13,14].

3.1 Flavonoids detection

(sodium Hydroxide 10% test)

Three drops of 10% NaOH were put to a test tube containing a little quantity of the extract for this experiment. The existence of flavonols was established by the emergence of purple-red and yellow-orange ppt[15,16].

3.2 Detection of alkaloids

A dosage of 15 mg of the extract was dissolved in 2 mL of 5% HCl. After being appropriately combined, this material was screened. The filtrate was divided into 4 separate tubes, and each tube was treated with a different reagent (Mayer's, Wagner's, Dragendorff's, and Bouchardat's). Alkaloids were evident in the creation of the yellowish-white Mayer's, brown Bouchardat's, red-orange Dragendorff's, and red-brown Wagner's ppt[3,12].

3.3 Detection of saponins (foam test)

20 drops of isopropyl alcohol, 2mL of water, and a few drops of olive oil were added to a little amount of extract to dissolve it. A Foamy layer was formed which confirmed the presence of saponins[17].

3.4 Detection of glycosides

3.4.1 Keller-killani test

In a test tube, 10mL of dissolved extract was taken. To this extract, some drops of 2% $FeCl_3$, 1.5 mL of concentrated sulphuric acid and4mL of glacial aceticacid were added. Glycosides were confirmed by the formation of the brown ring in between the layers[17].

3.4.2 Salkowski's test

A 2mL solution of concentrated sulfuric acid and distilled water was used to dissolve the trace quantity of plant extract. The emergence of a reddish brown colour served as confirmation that the steroidal aglycone portion was present[9].

3.5 Determination of tannins

A test tube was filled with around 1 mL of the dissolved extract, 2 mL of distilled water, and 4-5 drops of 10% ferric chloride solution. Tannins were present, as shown by the appearance of a greenish-blue colour[18].

4.Antioxidant Assay

Methodology:

4.1 Evaluation of free radical scavenging potential

Using the DPPH radical scavenging assay and the ABTS•+ radical cation decolorization test using trolox as the standard, the extract's ability to scavenge free radicals was assessed**.**

4.2 ABTS$^{\bullet+}$ radical scavenging potential

The approach described by Re et al. (Re et al., 1999) was followed in order to determine the ABTS•+ radical scavenging potential. The ABTS•+ radical cation was produced by adding 2.45 mM potassium persulfate to a 7 mM ABTS aqueous solution. This radical cation was then allowed to stand for 12 to 16 hours at room temperature and in the dark. The working solution, which had an initial absorbance of approximately 0.70 0.02 at 745 nm, was made by diluting the stock solution with ethanol. By mixing different concentrations of the sample (5 g/mL to 100 g/mL) and standard ascorbic acid (5 g/mL to 50 g/mL) with the reference solution ABTS+ to create a final volume of 1 mL, the free radical scavenging potential was calculated. At 30°C, the absorbance was meticulously measured after 6 minutes. The half-maximal inhibitory concentration (IC50) for test compounds and ascorbic acid was obtained by plotting the scavenging capability versus concentration[13].

4.3 1, 1-Diphenyl-2-Picrylhydrazyl radical-scavenging potential

With the use of a DPPH radical-scavenging method described in Brand-Williams et al. (1995), the samples' potential to scavenge free radicals was assessed. In 70% methanol, a 0.2 mM concentration of DPPH solution was created and stirred at room temperature for a whole night. By mixing ascorbic acid standards (5 g/mL to 50 g/mL) and samples (10 g/mL to 200 g/mL) in a final volume of 1 mL with DPPH solution, their capacity to scavenge free radicals was calculated. These samples were well shaken and kept at room temperature for 30 minutes in the dark. The reduction in absorbance for test materials and ascorbic acid after 30 minutes at 517 nm was seen (at room temperature), and ascorbic acid's concentration was calculated by plotting the scavenging capability against the concentration[19].

4.4 Antioxidant activity using DNA nicking assay

DNA damage protection assay was used to evaluate the antioxidant activity of extracts (methanol extract) rich in bioactive polyphenols. Supercoiled pBSK plasmid DNA was used for the analysis, which was carried out using the Zhao et al. (2005) method with a few minor adjustments. Plasmid DNA (0.5 µg) and extracts (50 µg/mL) were combined, and the mixture was incubated at room temperature for 10 min. Fenton's reagent (30 mM H_2O_2, 80 mM $FeCl_3$, and 50 mM ascorbic acid) was then added in an equivalent amount. The following step was to incubate reaction mixtures for 30 minutes at 37°C. On a 1% agarose gel, the DNA was analyzed using ethidium bromide staining. Curcumin was used as a positive control[20].

5 Results:

5.1 Qualitative screening:

The results of qualitative screening showed the presence of various biomolecules in all extracts viz n-hexane extract, chloroform extract, aqueous and methanolic extract. Biomolecules include

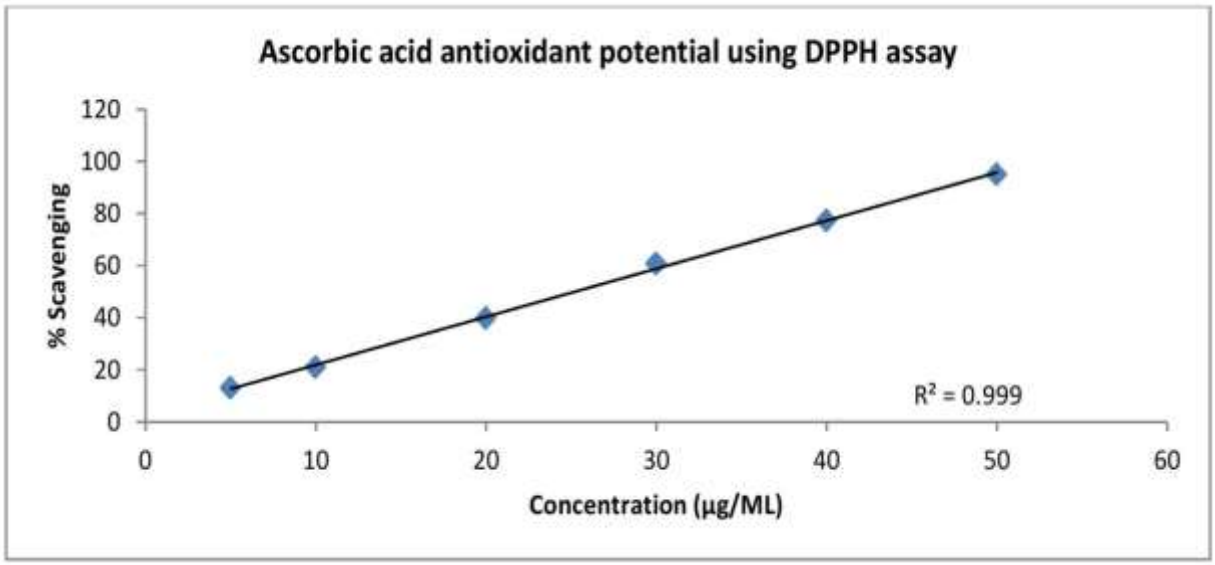
alkaloids,phenol, saponins, protein, glycosides, tannins, carbohydrate and steroids. The following table shows the presence of various constituents

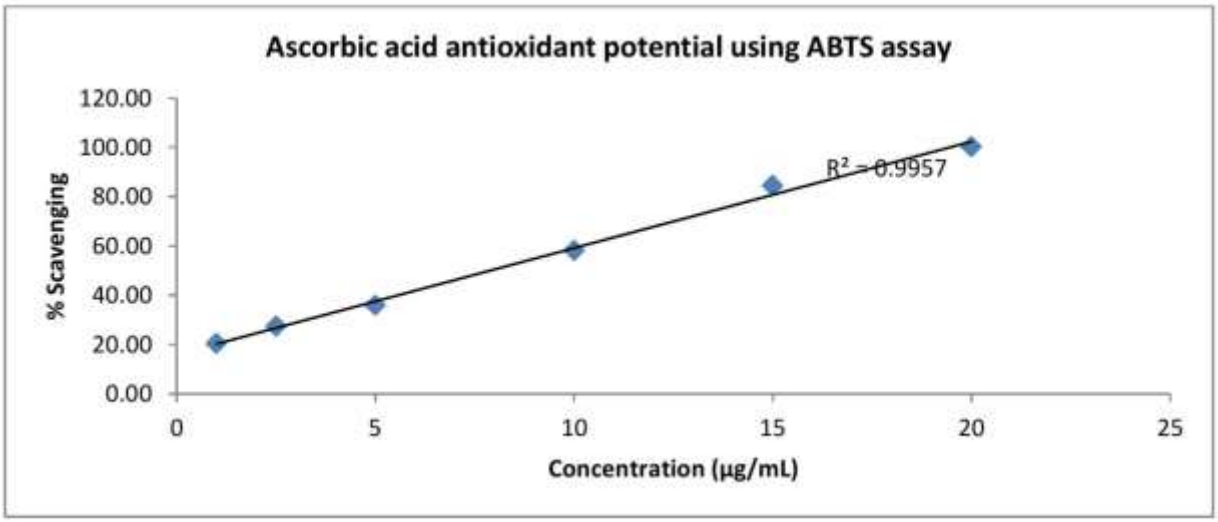
Sr. No	Phytochemicals Test	. Methanolic Extract	n-hexane extract	Chloroform extract	Aqueous extract
1. Alkaloids	a). Mayer's Test	+++	+	++	++
	b). Dragondroff's Test	+	+	+	+
2. Steroids	a). Salkowski's Test	++	+	-	+
	b). Liebermann's Burchard's Test	++	+	++	+
3. Phenols	Phenol Test	+++	-	+	++
4. Flavones	a). Aqueous Test	++	-	+	++
	b). H2So4 Test	+++	+	+	++
5. Tannins	Ferric chloride Test	++	-	+	-
6. Saponins	Aqueous Test	+++	+	+	++
7. Glycosides	a). H2So4 Test	+++	-	+	+
	b). Kellar Kilani Test	++	-	+	+
8. Proteins and Amino Acids	Millon's Test	+++	-	++	+
9. Carbohydrates	Molisch Test	++	-	++	+

Table 1 Qualitative analysis of various extracts of *Cinnamon tamala* leaf

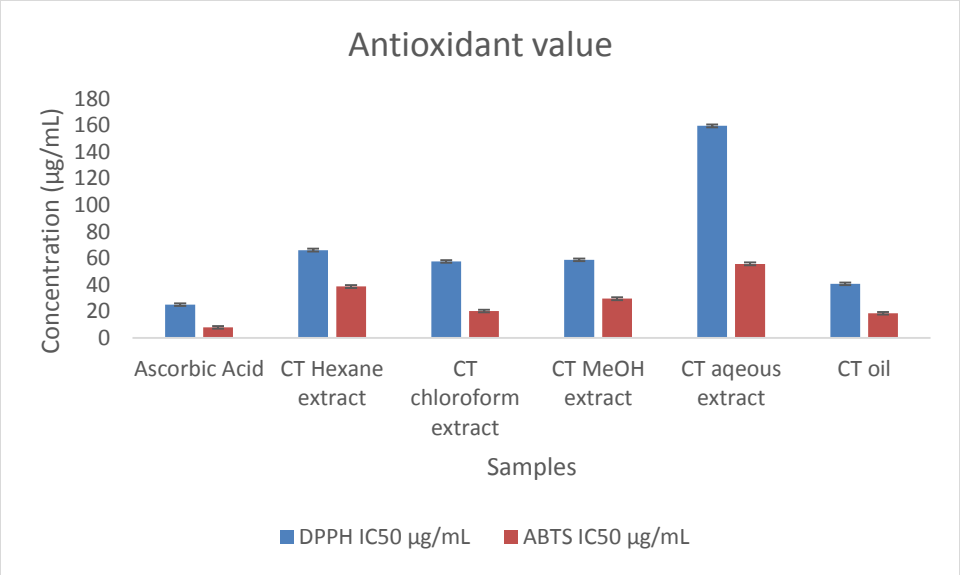
5.2 Free radical scavenging potential

The phytochemical nature of different extracts might influence the method used to analyses the antioxidant capacity of sample and accordingly two methods namely $ABTS^{\bullet+}$ and DPPH were used to understand the comprehensive antioxidant potential of different extracts. The results of antioxidant capacity of various extracts of sample Cinnamon *Tamala* evaluated by $ABTS^{\bullet+}$ and DPPH are summarized in **Table 2.** Cinnamon oil exhibited highest antioxidant activity having IC_{50} value of 40.85± 4.96 and 18.57 ± 0.10µg/mL in DPPH and ABTS assay. Among the various solvents used in sample extraction, chloroform extract exhibits higher antioxidant potential having IC_{50} value of 57.56 ± 0.57µg/mL and 20.32 ±0.21µg/mL in DPPH and ABTS assay.





Sample	DPPH IC_{50}µg/mL	ABTS IC_{50}µg/mL
Ascorbic Acid	25.20 ± 0.23	8.02 ± 0.13
CT Hexane extract	66.18 ± 0.40	38.76 ± 0.38
CT chloroformextract	57.56 ± 0.57	20.32 ±0.21
CT MeOH extract	58.77 ± 3.72	29.61 ± 0.41
CT aqeous extract	159.67 ± 2.67	55.80 ± 0.40
CT oil	40.85± 4.96	18.57 ± 0.10



5.3 Antioxidant potential using DNA nicking assay:

Considering the destructive potential of hydroxyl radicals, DNA damage protective activity was evaluated using DNA nicking assay. The plasmid DNA in the native super coiled form (Type I, Lane 1) was degraded into nicked single-stranded and double-stranded linear DNA (Type II and III, Lane 7) by hydroxyl radicals produced by Fenton's reaction mixture (Figure).The presence of standard curcumin (25 µg/mL) (Lane 3) protected the super coiled shape of DNA. In a similar way, the presence of 50 µg/mL quantities of chloroform, hexane, MeOH, and cinnamon oil in the reaction mixture reduced DNA damage.

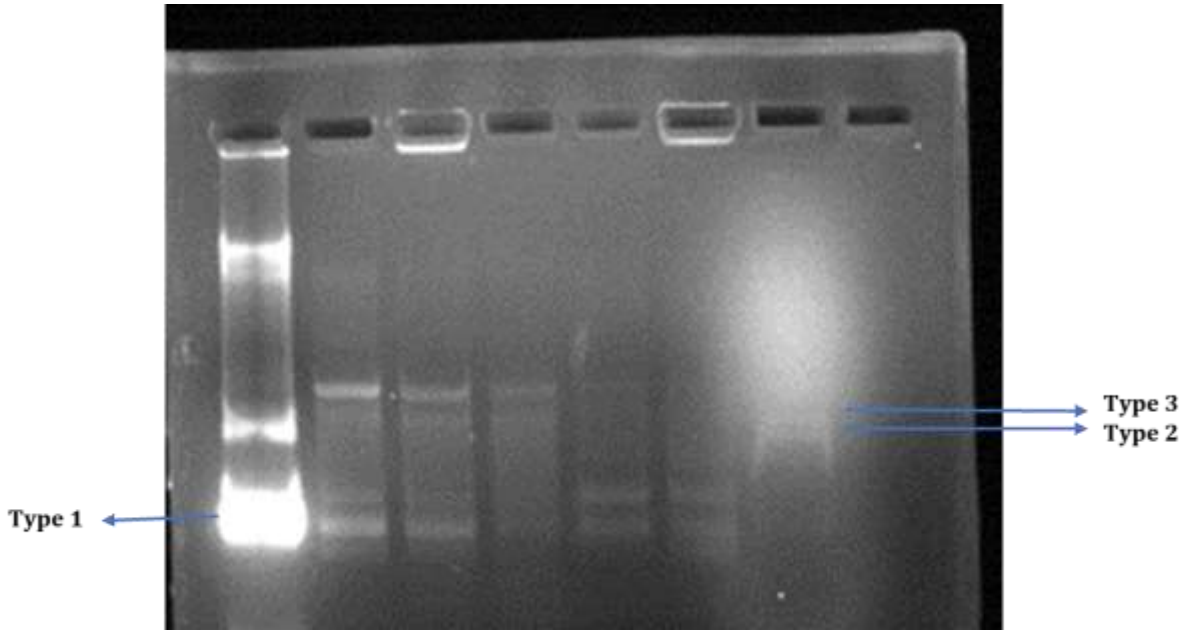


Figure : DNA Protective activity of cinnamon sample. Lane 1: Native plasmid DNA pBSK (without treatment); Lane 2: 50µg/mL Curcumin along with DNA treated with Fenton's

reagents; Lane 3-6 Cinnamon oil, hexane extract, MeOH extract and chloroform extract (25 µg/mL,) along with DNA treated with Fenton's reagents and Lane 7: Nicked DNA after treatment with Fenton's reagent

Conclusion: The results of this investigation demonstrated the presence of many phytochemicals in the leaf methanolic extract of *Cinnamomum tamala*, including polyphenols, flavonoids, flavones, and flavonols, alkaloids, steroids, tannins, and glycosides. Alkaloids already have antifungal, antibacterial, anticancer, and spasmolytic properties. Additionally, it was discovered that leaf extract had a phenolic concentration that was higher than that of other Lauraceae species but lower than that of other therapeutic plants.

The majority of phenolic compounds have the ability to absorb free radicals and neutralise them, delaying the ageing process of our cells. Additionally, *Cinnamomum tamala's* higher phenol concentration may have some anticancer properties. Compared to other members of the Lauraceae family, it demonstrates antioxidant activity and DPPH free radical scavenging ability. As a result, it is inferred that the polyphenols and flavonoids present in Cinnamomum *tamala* give rise to its therapeutic qualities. It had a greater concentration of polyphenols, was a rich source of antioxidants, and had free radical scavenging properties. Consumption of dietary supplements high in polyphenols has been linked to a number of health advantages, including the improvement of organ malfunction and health-related concerns. the potential for interactions with other bioactives found in herbal remedies or in dietary supplements.

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