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# Phytochemical Profiling and Antioxidant Activity of *Cinnamomum tamala* Methanolic Leaf Extract

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**ABSTRACT**

**Background:** *Cinnamomum tamala*, Indian bay leaf, also known as tej patta, is a tree that belongs to Lauraceae family which is native to India. It is well known for its antitumor, anticancer, anti-inflammatory and other bioactivities. **Methods:** Present study was designed to evaluate the qualitative and quantitative phytochemical profile of leaf extract. Following the phytochemical screening (qualitative and quantitative) leaf extract was examined for antioxidant free radical scavenging potency (DPPH, FRAF and ABST assays). **Results:** qualitative phytochemical assessment revealed the presence of polyphenols, flavones, alkaloids, steroids, terpenoids and glycosides. Quantitative assessment revealed that leaf extract contains polyphenolic content 44.5 mg GAE/g, flavonoids content 23.8 (mg QE/g) and total flavones and flavonols 0.67 (mg QE/g). The DPPH free radical assay revealed that *Cinnamomum tamala* methanolic extract at a 100µm/ml concentration showed the % inhibition activity 92.29± 0.89. Vitamin C equivalent antioxidant capacity (VCAC) was measured by using ABTS and FRAP method. Results for leaf extracts was 103.05 for ABTS mg Vitamin C equivalent (VC)/g respectively for the ABTS method. And for FRAP assay, results for leaf extracts were 92.34 mg VC/g respectively. **Conclusion:** Considering the findings of present study it can be concluded that *Cinnamomum* leaf extract may used as food supplement and alternative therapy for the treatment of various diseases.

## INTRODUCTION

Plant parts can be used for the treatment of diseases, known as medicinal plants. Herbal drugs are derived from different plant parts with low cost and easy availability. It may include entire parts of plants such as leaves, roots, bark, flower and seeds of plants. In India, plants are widely used in folk medicine as well as the pharmaceutical industry. Folk medicine is used in the different native system of medication such as Siddha, Ayurveda and Unani etc. India have rich source of plant species estimated about 4.5 million. But still, 2.5 lakh to 5 lakh plant species have been identified and investigated for biological and pharmacological purpose [1]. Secondary metabolites may be used for the treatment of various diseases, and in the form of extract it would be used as a novel preparation of drugs in pharmacological industries [2]. These medicinal or aromatic plants can play a significant role in the enhancement of employment between rural people [3]. According to the World Health organization (WHO) report, 80% population in India depends upon traditional medicine for their initial healthcare necessities. Since, it is proved as effective and low in cost with lesser side effects [4]. Natural herbs have been highly valued source of medicine throughout the human history and indicating a growing part of modern high-tech medicine. The herbal drugs are formulated commercially because of its easy availability, medicinal properties and its fewer side effects [5]. Though, medicinal plants consequential medicines that called as well-defined herbal drugs such as quality and availability of raw materials, standardization and stability of quality control. Herbal medicines are natural occurring, easily available with cost-effective and have minimum side effects. Most of the herbal plants have medicinal properties which are initially derived from plant parts. Therefore, these herbs are best alternative with fewer side effects.

Since ancient era, medicinal plants and herbs have been vital part of traditional medicine. India has primordial inheritance of traditional medicine distinguished by Ayurveda, Siddha and Unani system. The use and consumption of plants and natural products have played fundamental roles in human health care. The age-old system of herbal medicine and natural products is being revived by day-to-day practice for its long-lasting curative effect, easy availability and natural way of healing with lesser side effects [6]. Nature is rich with diversified natural compounds that can be used as the discovery of new drugs [7]. Medicinal plants have been used as a flavouring agent, food preservation and to treat health diseases/disorders. Plants are source of various secondary metabolites and reliable for biological activities. Natural products with miscellaneous bioactivities are becoming an

important source of novel agents and have diverse therapeutic potential. Most of the currently available drugs were obtained from natural compounds [9-11]. Various bioactive compounds are found in plants such as polyphenols, flavonoids, flavones, alkaloids, saponins and glycosides. Natural antioxidants consumed by humans especially flavonoids have gained much interest as a mean to prevent oxidative damage under various pathological conditions by inhibiting the production of free radicals, antibacterial, hepatoprotective, anti-inflammatory, anticancer and antiviral agents. Different medicinal plants are present locally in different areas which are unexplored such as *Aslilium polyphyllum, Achyranthes bidentate, Cinnamomum tamala, Ageratum conyzoides, Berberis glaucocarpa* and *Capsella bursa-pastoris* etc.

*Cinnamomum tamala*, Indian bay leaf, also known as tej patta, is a tree belongs to Lauraceae family which is native to India. This family contains about 55 genera and over 2000 species worldwide, mostly from tropical regions. Historically, it is one of the oldest known and used species. It is an evergreen tree up to 8m in height and widely used in pharmaceutical uses [12-13]. Tej patta is generally used as spice, but few studies reported that tej patta possess various pharmacological activities such as anti-hyperlipidemic activity, anti-diabetic activity, gastro protective activity, anti-helminthic/ antiprotozoal activity, anti-inflammatory property, antiemetic activity, anti-diarrhoeal, antifungal activity, free radical scavenging activity also exhibit central nervous system (CNS) protective activity like anticonvulsant, hypothermic activity. The extract of plant reduces the oxidative damage and is useful for the protection against renal toxicity. It is useful to treat various diseases such as anal and rectal disease, flatulence, cancer, coryza (inflammation in mucous membrane), anorexia, liver and spleen diseases, cardiovascular diseases [14-16]. Therefore, the present study was designed to characterize the phytochemical profile of *Cinnamomum tamala* methanolic extract of leaf.

## MATERIALS AND METHODS

### *Chemicals and reagents*

Methanol, ethanol, hydrochloric acid (HCl), isopropyl alcohol, glacial acetic acid, chloroform, conc. sulphuric acid ($H_2SO_4$), sodium hydroxide (NaOH), ferric chloride ($FeCl_3$), sodium carbonate ($Na_2CO_3$), gallic acid, ammonium hydroxide ($NH_4OH$), sodium nitrite ($NaNO_3$), aluminium chloride ($AlCl_3$), quercetin. All chemicals were use of an analytical mark. Ferric chloride hexahydrate ($FeCl_3.6H_2O$), [(2,20-Azino-bis(3-ethylben- zothiazoline-6-sulfonic acid) diammonium salt] (ABTS+), 1,1-Diphenyl-2-picryl hydrazyl (DPPH),2,4,6-

tri(2-pyr- idyl)-s-triazine (TPTZ), sodium carbonate, sodium acetate, vitamin C (ascorbic acid), gallic acid, quercetin, mercuric chloride, trichloroacetic acid, Folin-Ciocalteu phenol reagent, hydrochloric acid, sulphuric acid, iodine, sodium carbonate, aluminum trichloride, potassium acetate, Dragendorff's reagent, potassium iodide were purchased from Sigma–Aldrich. Ethanol, methanol, chloroform, ammonia, glacial acetic acid, sodium hydroxide (NaOH) and potassium peroxodisulfate were purchased from Merck.

*Procurement of leaves and extract preparation*

*Cinnamomum tamala* (Tej Patta) leaves were collected from Jogindernagar, Himachal Pradesh and plant leaves were identified by a botanist from the department of Botany, Career Point University (Hamirpur). Leaves were washed in potassium dichromate followed by washing in distilled water to make the plant dust free. Then leaves were allowed shade dried and crushed by using mixer to make fine powder. The extract was prepared in methanol solvent (70%) by using Soxhlet apparatus at 60$^{o}$C. The dried extract was stored in the refrigerator at 4$^{o}$C till further use.

**Phytochemical (qualitative and quantitative) screening**

Crude extract of *Cinnamomum tamala* was evaluated for the presence or absence of various phytochemicals such as polyphenols, flavonoids, flavones, flavonols, saponins, terpenoids and carbohydrates by using standard methods. Based on color change and formation of precipitate was detected that used as marker of positive response to these phytochemical tests.

*Detection of alkaloids*

About 15mg extract was dissolved in 5% of 2ml hydrochloric acid and shaken to mix content properly and then filtered. In the filtrate, few drops of Mayer's reagent, Wagner's reagent, Bouchardat's reagent and Dragendorff's reagent were added in each tube. Formation of yellowish-white (Mayer's), red-brown precipitate (Wagner's), Brown precipitate (Bouchardat's) and red-orange precipitate (Dragendorff's) revealed the presence of alkaloids.

*Detection of flavonoids*

*Sodium hydroxide (10%) test*

A minute quantity of extract was taken in a test tube and add three drops of 10% sodium hydroxide (NaOH) in which the production of yellow-red, coffee-orange, purple-red color showed the presence of flavones and flavonols.

*Determination of saponins by foam test*

A minute quantity of extract was dissolved in 2ml of distilled water and 20 drops isopropyl alcohol, then shake. Few drops of olive oil were added then mixed. The formation of foamy layer showed the presence of saponins.

*Determination of glycosides*

*Liebermann's test*

A minute quantity of plant extract was taken in a test-tube, in which 2ml of chloroform and acetic acid was added respectively. The whole reaction mixture was allowed to cool and then few droplets of concentrated sulphuric acid ($H_2SO_4$) were added. Production of green colour indicated the presence of aglycone (steroidal part of glycosides).

*Keller-killiani test*

Approximately 10ml of dissolved (distilled water) extract was taken in a test-tube, in which 4 ml of glacial acetic acid, few drops of 2% $FeCl_3$ and 1.5ml of concentrated $H_2SO_4$ were added. Formation of brown ring between layers indicated the presence of glycosides.

*Salkowski's test*

A small amount of plant extract was dissolved in distilled water, in which 2.5ml of concentrated $H_2SO_4$ was added. Formation of reddish-brown color revealed the presence of steroidal aglycone part of the glycosides.

*Determination of tannins*

Approximately 1ml of dissolved (70% ethanol) was taken small in a test-tube, in which 2ml of distilled water was added and then 4-5 drops of ferric chloride ($FeCl_3$) solution (10%). Production of blue or green colour showed the presence of tannins.

**Quantitative assessment of phytochemicals**

*Assessment of total polyphenolic content*

The total phenolic was determined by the following method of Folin-Ciocalteu method [17]. About 22mg of extract was taken in a test tube, in which 66ml of distilled water were added into it. Then 50µl of diluted Folin-phenol reagent (10%) and 2.5ml of 20% sodium carbonate ($Na_2CO_3$) was added in each test tube. The whole reaction was shaken properly and then incubated under dark condition for 40 minutes. Following the incubation, absorbance was

taken at 725nm. The total phenolic content was quantified by plotting a standard curve using different concentrations of gallic acid as standard (mg GAE/g).

*Determination of flavonoids content*

Flavonoids were quantified by the following method of Zhishen *et al.*, (1999) [18]. Approximately 22gm of extract was dissolved in 66ml of 70% ethanol. 5% sodium nitrite ($NaNO_2$) solution was added in each test tube and then incubated at room temperature for 10 minutes. About 2ml of 4% sodium hydroxide solution and final volume upto 5ml by adding distilled water. The reaction mixture was shaken vigorously, then again incubated for 20 minutes at room temperature and absorbance was recorded at 510 nm (mg QE/g).

*Determination of flavones and flavonol content*

Flavones and flavonol content was quantified by the following method of aluminum chloride method [19] (Cvek *et al.*, 2007). About 22mg of plant extract was dissolved in 66ml of ethanol (70%), in which 0.2ml of aluminum chloride ($AlCl_3$), and 2.8ml of glacial acetic acid were added to make final volume upto 5ml in each test tube. The entire reaction mixture was incubated for 30 minutes at room temperature and absorbance was recorded at 415nm. The total flavone and flavonol content were determined by plotting a standard curve using various concentrations of quercetin (mg QE/g).

**Antioxidant assay**

**(i)** *Antioxidant activity: DPPH free radical scavenging assay*

The method of Blois, 1958 was used to estimate the free radical scavenging activity. 2, 2-diphenyl-1-picryl-hydrezyl (DPPH) was used to determine free radical scavenging activity [20]. About 0.2mmol/l solution of 2, 2-diphenyl-1-picryl-hydrezyl was prepared in methanol, and different concentrations of methanolic leaf extract *Cinnamomum tamala* (50-250$\mu$g/ml) were prepared in separate tubes, and 500$\mu$l of DPPH solution was added to each tube. In all the tubes reaction mixture was shaken and then allowed to stand as such for 30minutes at room temperature. Control solution (Group-I) was prepared in the same way without the addition of *Cinnamomum tamala* methanolic extract of leaf and ethanol were used for baseline correction. The absorbance was determined at 517nm by using a spectrophotometer. The decrease in absorbance revealed that there was the increase in free radical scavenging activity. Vitamin C (ascorbic acid) was used as a standard to compare the results. The potential of 2, 2-diphenyl-1-picryl-hydrezyl free radical scavenging activity was calculated

as

$$\text{DPPH scavenging activity (\% of inhibition)} = \frac{(Ao-A1)}{Ao} \times 100$$

Where,

Ao is the absorbance of the control

A1 is the absorbance of the sample extract

**(ii)** *BY ABST assay*

The free radical scavenging activity was assessed by using ABTS+ solution by following method [21-22]. The stock solution was prepared by mixing of ABTS solution and 2.6M m potassium persulphate (ratio 1:1) and allowed to react for 12h at room temperature und er dark conditions. The $ABTS^{+}$ solution was prepared by 3ml stock solution and absorbance was recorded at 734nm in a spectrophotometer. Standard solutions were separately prepared ranging from 0-130µg/ml. Sample extract and standard solutions (130µl) were kept in different test tubes under dark conditions (30minutes). Absorbance of both the samples were recorded at 734nm. The % inhibition and concentr ation of ABTS content was calculated. Ascorbic acid was used as standard.

**(iii)** *By ferric reducing antioxidant power (FRAP) assay*

The FRAP was assessed by following method of Gan *et al.*, (2010) [23]. 100ml of 300m M acetate buffer, 10ml of 10mM TPTZ solution and 10ml of 20mM $FeCl_3.6H_2O$ was used to prepare FRAP reagent and incubated at 37ºC. Acetate buffer was prepared as follows: sodium acetate trihydrate (3.1g) was dissolved in distilled water (500ml) and then glacial acetic acid (16ml) was added and volume made up upto 1litre. 10mM TPTZ solution was prepared in 40mM HCl and 20mM $FeCl_3.6H_2O$ was prepared in distilled water. Standard solutions were separately prepared ranging from 0-150µg/ml. Sample extract and standard solutions (150µl) were kept in different test tubes under dark conditions (30minutes).

Absorbance of both the samples were recorded at 593nm. The concentration of FRAP co ntent was equivalent as ascorbic acid (standard). ABTS content was calculated.

## RESULTS AND DISCUSSION

## RESULTS

### Phytochemical (qualitative and quantitative) screening

*Qualitative analysis*

The qualitative phytochemical screening showed that leaf extract contains biomolecules including alkaloids, tannins, saponins, phenols, glycosides, proteins, steroids and carbohydrates.

*Quantitative assessment of phytochemicals*

*Total polyphenolic content*

The total polyphenolic content was estimated in the leaf extract which showed that phenolic content increases by concentration varies between 0.3mg/ml to 3mg/ml. However, the higher polyphenolic content was recorded at 3mg/ml (Figure 1).

*Total flavonoids content*

The total flavonoids content was quantified in the leaf extract which showed that total flavonoids content increases in concentration-dependent manner. All the concentrations were selected on the basis of preliminary studies, by taking concentration above 3mg/ml and below 0.3mg/ml. Therefore, concentrations between 0.3mg/ml-3mg/ml were selected based on absorbance range. The higher flavonoids concentration was observed in 3mg/ml (Figure 2).

*Total flavones and flavonol content*

The total flavones and flavonols were estimated in the leaf extract which exhibit higher concentration of extract increases the flavones and flavonol content. These concentrations were selected based on preliminary studies (0.3mg/ml-3mg/ml). Therefore, concentrations were selected based on absorbance range and maximum concentration absorbed at 3mg/ml (Figure 3).

*Cumulative quantitative screening*

The cumulative quantitative screening revealed that leaf extract contains various essential bioactive compounds such as polyphenols, flavonoids, flavones and flavonols. However, the

concentration of these bioactive compounds varies polyphenols (44.5mg/g), flavonoids (23.8 mg/g), flavones and flavonol (0.67 mg/g) (table 2).

*Assessment of antioxidant activities*

The antioxidant ability and free radical scavenging are related with the properties of plants. In the current, the antioxidant activity of *C. tamala* leaf extract was measured using three different assays, such as DPPH, FRAP and ABTS. Evaluation of single antioxidant assay could not give the correct result, because the antioxidant activity of plant extract is affected by various, for example the test system and composition of extract. Hence, it is essential to conduct more than one type of antioxidant potential assessment to cover the different mechanisms of antioxidant actions (Gan *et al.*, 2010). Ferric reducing antioxidant power (FRAP) assay depends of the reduction of ferric ions into ferrous ion (Benzie and Strain, 1996). DDPH is nitrogen centered free radical possessing an odd electron which gives a strong absorption at 517nm, its color changes from purple to yellow when DPPH odd electron is paired off in the presence of radical scavenger to produce the reduced DPPH-H (Cai *et al.*, 2003). ABTS assay depends on the antioxidant compound potential to scavenge ABTS radical. This antioxidant assay can be used to examine the antioxidant potency of lipophilic and hydrophilic compounds. The entire three assays are very simple, cost-effective and are generally used for the evaluation of antioxidant activity and can provide same results. Figure shows the comparative account of three assays performed in *Cinnamomum tamala* methanolic extract of leaf.

DPPH *free radical scavenging assay*

DPPH free radical scavenging activity of *Cinnamomum tamala* leaf extract was observed as shown in figure 5 and Table 3. The methanolic leaf extract of *Cinnamomum tamala* at various concentrations ranged from 1 to 92*µ*g/ml was estimated with vitamin C (ascorbic acid) as a standard. Figure 5 shows the % inhibition as a function of vitamin C concentration. The results exhibited the DPPH radical scavenging activity of Methanolic leaf extract of *Cinnamomum tamala* at various concentrations as shown in Table 3 and figure 5. In *Cinnamomum tamala* hydroalcoholic leaf extract at a 92µm/ml concentration the % inhibition activity was 92.29± 0.89.

## DISCUSSION

Plants are very good sources of bioactive components (flavonoids, polyphenols, flavones, carbohydrates and glycosides etc.) present in medicinal plants. Phytochemicals are natural bioactive compounds that are recognized as physiological as well as medicinal properties. In the present study, *Cinnamomum tamala* leaf extract was prepared in 70% v/v methanol. Phytochemical screening (qualitative analysis) revealed the presence of alkaloids, steroids, carbohydrates, tannins, saponins, glycosides, proteins, amino acids, phenols and flavones in *Cinnamomum tamala* methanolic extract of leaf. All these biomolecules are essential for various metabolic processes, moreover, plant-derived secondary metabolites such as alkaloids, tannins, saponins, and polyphonic compounds are having therapeutic efficacy.

The phytophenolic compounds present in the *C. tamala* extract are very well known having medicinally important. Such as alkaloids are reported as strong poisons and various alkaloids derived from medicinal plants exhibits biological functions such as anti-inflammatory [24], antimalarial [25], antimicrobial [26], cytotoxicity, antispasmodic and pharmacological ef fects [27-28]. Likewise, steroids derived from plants are essential cardiotonic effects and also possess antibacterial and insecticidal properties. Sometimes they are used in the formulation of medicines due to their well-known biological activities, tannins are having antibacterial [29], antitumor and antiviral activities [30]. Glycosides are used for the treatment/therapy of congestive heart failure and cardiac arrhythmia [31]. These glycosides suppresses the $Na^+/K^+$ pump and increase the $Ca^{2+}$ level which elevates the contraction of heart muscles and improves cardiac output [32-33].

These bioactive components present in the *Cinnamomum tamala* leaf methanolic extract may showed biological properties. Therefore, on the basis of qualitative phytochemical screening leaf extract was preceded for quantitative phytochemical analysis. Alkaloids exhibited antimicrobial, anti-diarrheal, anthelminthic activities and act as inhibitor or growth terminator [34-35]. In addition, flavonoids showed antiviral, antioxidant and anti-inflammatory activities. Also, flavonoids have a chemo-preventive role in cancer across the effects on signal transduction in cell proliferation and angiogenesis. Whereas tannins act as a good antioxidants and free radical scavengers [36]. Similarly, tannins and terpenoids also plays vital role in antioxidant mechanism that works synergistically with each other to make extensive variety of antioxidative actions. Plant polyphenols compound retain aromatic benzene with hydroxyl group an aromatic benzene ring having capability to absorb free

radicals to chelate metal ions. It may be catalyse the reactive oxygen species production and lipid peroxidation level [37]. Therefore, it can be suggested that polyphenolics, flavonoids may work in additive or synergistic manner with other phytochemicals present in *C. tamala* and make it medicinally important.

*Cinnamomum tamala* methanolic extract (CTME) were assessed for quantitative analysis of the total polyphenolic content, total flavonoids content, total flavones and flavones content. Phenols are ranging from simple to complex having aromatic or polymeric structures which regularly endure in the glyosidic forms [38]. It plays important role in metabolism and is abundantly found in plants (Pal and Verma, 2013). Findings of the present study revealed that the polyphenols are present in higher amount in *Cinnamomum tamala* methanolic leaf extract. However, total polyphenolic content, total flavonoids content, total flavones and flavonols content were quantified in the leaf extract which exhibited that content increases with an increase in the concentration of extract (3mg/ml). Therefore, the concentration of these bioactive compounds varies polyphenols (44.5mg/g), flavonoids (23.8 mg/g), flavones and flavonol (0.67 mg/g) (table 2).

Evaluation of antioxidant activities (FRAP, ABTS and DPPH) revealed that *C. tamala* possesses significant antioxidant properties. Principal compound present in *C. tamala* methanolic extract of leaf was polyphenols, our results exhibited that the phenolic content is higher in leaf extract. Therefore, leaf extract can be used as a source of antioxidants, this antioxidant activity might be due to their redox potential which contributes considerably neutralize the free radicals and quenching oxygen molecule [39].

## CONCLUSION

The result of this study showed the presence of some phytochemicals such as polyphenols, flavonoid, flavones and flavonols, alkaloids, steroids, tannins and glycosides in leaf methanolic extract of *Cinnamomum tamala*. Alkaloids are already kno wn to have spasmolytic, antifungal, antimicrobial and antitumor activities. The findings also revealed that leaf extract have phenolic content greater than other Lauraceae species but lower than another medicinal plant.

Mostly, phenolic compounds can capture free radicals and neutralize them thus preventing our cells from aging process. Moreover, greater phenolic content in *Cinnamomum tamala* may exhibit some anticancer activities. It exhibits DPPH free

radical scavenging capacity and antioxidant activity in comparison with the other Lauraceae family. Therefore, it is concluded that *Cinnamomum tamala* shows medicinal properties due to the presence of polyphenols and flavonoids. It showed higher amount of polyphenols, good source of antioxidant and exhibits free radical scavenging activity. Consumption of polyphenol-rich food supplements has been associated with a range of health benefits such as improving health-related issues and organ dysfunction. The possibility of interactions with another bioactive present in herbal medicine or that can be administered as supplements. This is a current study and more work is being carried to explore its biological activities.

## Abbreviations

DPPH: 2, 2-diphenyl-1-picryl-hydrezyl, FRAP: ferric reducing antioxidant power, GAE: Gallic acid, QE:Quercetin, CNS: central nervous system, CTME*: Cinnamomum tamala* methanolic extract,

## Author's contribution

YAH and PS has conceived the experiment, PS and RR has conducted the experimental study. PS has drafted the manuscript, PS and YAH has interpreted the results. YAH, RK and RAB have proofread the manuscript. All authors have approved the manuscript.

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## Conflict of interest

The authors declare there is not any conflict of interest

## REFERENCES

1. Srinivasan, N., 2018. Pharmacognostical and phytochemical evaluation of Cassia alata Linn. *J Med Plants*, *6*, pp.69-77.
2. Singh, P., Singh, R., Sati, N., Ahluwalia, V. and Sati, O.P., 2017. Phytochemical and pharmacological significance of genus: Impatiens. *Int. J. Life. Sci.Scienti. Res*, *3*(1), pp.868-881.
3. Sharma, P., Kumar, P., Sharma, R., Gupta, G. and Chaudhary, A., 2017. Immunomodulators: Role of medicinal plants in immune system. *National Journal of Physiology, Pharmacy and Pharmacology*, *7*(6), pp.552.

4. Odhav, B., Thangaraj, K., Khumalo, N. and Baijnath, H., 2013. Screening of African traditional vegetables for their alpha-amylase inhibitory effect. *Journal of Medicinal Plants Research*, *4*(14), pp.1502-1507.
5. Emerald, M. and Marrero, D., 2018. Medicinal Plants Research. In *Herbs for Diabetes and Neurological Disease Management*, pp.251-303.
6. Del Prete, M., Mauriello, M.C., Faggiano, A., Di Somma, C., Monfrecola, G., Fabbrocini, G. and Colao, A., 2012. Insulin resistance and acne: a new risk factor for men?. *Endocrine*, *42*(3), pp.555-560.
7. Gordaliza, M., 2007. Natural products as leads to anticancer drugs. *Clinical and Translational Oncology*, *9*(12), pp.767-776.
8. Newman, D.J. and Cragg, G.M., 2007. Natural products as sources of new drugs over the last 25 years. *Journal of natural products*, *70*(3), pp.461-477.
9. Umthong, S., Phuwapraisirisan, P., Puthong, S. and Chanchao, C., 2011. In vitro antiproliferative activity of partially purified Trigonalaevicepspropolis from Thailand on human cancer cell lines. *BMC complementary and alternative medicine*, *11*(1), pp.1-8.
10. Sforcin, J.M. and Bankova, V., 2011. Propolis: is there a potential for the development of new drugs?. *Journal of ethnopharmacology*, *133*(2), pp.253-260.
11. Vardar-Ünlü, G., Candan, F., Sökmen, A., Daferera, D., Polissiou, M., Sökmen, M., Dönmez, E. and Tepe, B., 2003. Antimicrobial and antioxidant activity of the essential oil and methanol extracts of Thymus pectinatusFisch. etMey. Var. pectinatus (Lamiaceae). *Journal of Agricultural and food chemistry*, *51*(1), pp.63-67.
12. Mehta, S., Purohit, V.K. and Andola, H.C., 2014. Pharmacological Activities of Cinnamomum tamala Nees&Eberm. and Medical Implication: A Review. *Medicinal & Aromatic Plants*, *3*(4), pp.1-6.
13. Tiwari, S. and Talreja, S., 2020. Importance of Cinnamomum Tamala in the Treatment of Various Diseases. *Pharmacognosy Journal*, *12*(6s).
14. Aggarwal, B., Prasad, S., Reuter, S., Kannappan, R., R Yadav, V., Park, B., Hye Kim, J., C Gupta, S., Phromnoi, K., Sundaram, C. and Prasad, S., 2011. Identification of novel anti-inflammatory agents from Ayurvedic medicine for prevention of chronic diseases:"reverse pharmacology" and "bedside to bench" approach. *Current drug targets*, *12*(11), pp.1595-1653.
15. Siddhuraju, P. and Becker, K., 2003. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (Moringaoleifera Lam.) leaves. *Journal of agricultural and food chemistry*, *51*(8), pp.2144-2155.
16. Zhishen, J., Mengcheng, T. and Jianming, W., 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food chemistry*, *64*(4), pp.555-559.
17. Cvek, J., Medić-Šarić, M., Jasprica, I., Zubčić, S., Vitali, D., Mornar, A., Vedrina-Dragojević, I. and Tomić, S., 2007. Optimisation of an extraction procedure and chemical characterisation of Croatian propolis tinctures. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques*, *18*(5), pp.451-459.
18. Blois, M.S., 1958. Antioxidant determinations by the use of a stable free radical. *Nature*, *181*(4617), pp.1199-1200.
19. Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L. and Byrne, D.H., 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of food composition and analysis*, *19*(6-7), pp.669-675.
20. Gan, R.Y., Xu, X.R., Song, F.L., Kuang, L. and Li, H.B., 2010. Antioxidant activity and total phenolic content of medicinal plants associated with prevention and treatment of cardiovascular and cerebrovascular diseases. *Journal of Medicinal Plants Research*, *4*(22), pp.2438-2444.
21. Benzie, I.F. and Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry*, *239*(1), pp.70-76.
22. Cai, Y., Sun, M. and Corke, H., 2003. Antioxidant activity of betalains from plants of the Amaranthaceae. *Journal of agricultural and food chemistry*, *51*(8), pp.2288-2294.
23. Souto, A.L., Tavares, J.F., Da Silva, M.S., Diniz, M.D.F.F.M., Athayde-Filho, D., Filgueiras, P. and Barbosa Filho, J.M., 2011. Anti-inflammatory activity of alkaloids: an update from 2000 to 2010. *Molecules*, *16*(10), pp.8515-8534.

24. Dua, V.K., Verma, G., Singh, B., Rajan, A., Bagai, U., Agarwal, D.D., Gupta, N.C., Kumar, S. and Rastogi, A., 2013. Anti-malarial property of steroidal alkaloid conessine isolated from the bark of Holarrhenaantidysenterica. *Malaria journal*, *12*(1), pp.1-6.
25. Benbott, A., Yahyia, A. and Belaidi, A., 2012. Assessment of the antibacterial activity of crude alkaloids extracted from seeds and roots of the plant Peganumharmala L. *Journal of Natural Product and Plant Resources*, *2*(5), pp.568-573.
26. Ameyaw, Y. and Duker-Eshun, G., 2009. The alkaloid contents of the ethno-plant organs of three antimalarial medicinal plant species in the eastern region of Ghana. *Int J ChemSci*, *7*(1), pp.48-58.
27. Thite, S.V., Chavan, Y.R., Aparadh, V.T. and Kore, B.A., 2013. Preliminary phytochemical screening of some medicinal plants. *Int. J. Pharm. Chem. Biol. Sci*, *3*(1), pp.87-90.
28. Graça, V.C., Ferreira, I.C. and Santos, P.F., 2016. Phytochemical composition and biological activities of Geranium robertianum L.: A review. *Industrial Crops and Products*, *87*, pp.363-378.
29. Jain, P., Sharma, H.P., Basri, F., Baraik, B., Kumari, S. and Pathak, C., 2014. Pharmacological profiles of ethno-medicinal plant: Plumbagozeylanica l.-A review. *Int J Pharm Sci Rev Res*, *24*(1), pp.157-63.
30. Chikezie, P.C., Ibegbulem, C.O. and Mbagwu, F.N., 2015. Medicinal potentials and toxicity concerns of bioactive principles. *Med Aromat Plants*, *4*(3), pp.1-15.
31. Banso, A. and Adeyemo, S., 2006. Phytochemical screening and antimicrobial assessment of Abutilon mauritianum, Bacopamonnifera and Daturastramonium. *Biokemistri*, *18*(1).
32. Aiyelaagbe, O.O. and Osamudiamen, P.M., 2009. Phytochemical screening for active compounds in Mangiferaindica leaves from Ibadan, Oyo State. *Plant Sci Res*, *2*(1), pp.11-13.
33. Tran, N., Pham, B. and Le, L., 2020. Bioactive compounds in anti-diabetic plants: From herbal medicine to modern drug discovery. *Biology*, *9*(9), p.252.
34. Saxena, M., Saxena, J., Nema, R., Singh, D. and Gupta, A., 2013. Phytochemistry of medicinal plants. *Journal of pharmacognosy and phytochemistry*, *1*(6).
35. Ginwala, R., Bhavsar, R., Chigbu, D.G.I., Jain, P. and Khan, Z.K., 2019. Potential role of flavonoids in treating chronic inflammatory diseases with a special focus on the anti-inflammatory activity of apigenin. *Antioxidants*, *8*(2), p.35.
36. Rajanandh, M.G. and Kavitha, J., 2010. Quantitative estimation of β-sitosterol, total phenolic and flavonoid compounds in the leaves of Moringaoleifera. *International Journal of PharmTech Research*, *2*(2), pp.1409-1414.
37. Williamson, G. and Manach, C., 2005. Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. *The American journal of clinical nutrition*, *81*(1), pp.243S-255S.
38. Pal, D. and Verma, P., 2013. Flavonoids: A powerful and abundant source of antioxidants. *International Journal of Pharmacy and Pharmaceutical Sciences*, *5*(3), pp.95-98.
39. Abdel-Wahab, S.I., Abdul, A.B., Zhew, K. and Fong, S.M., 2009. Antimicrobial and free radical scavenging activities of the dichloromethane extract of Goniothalamusumbrosus. *Int. J. Trop. Med*, *4*(1), pp.32-36.

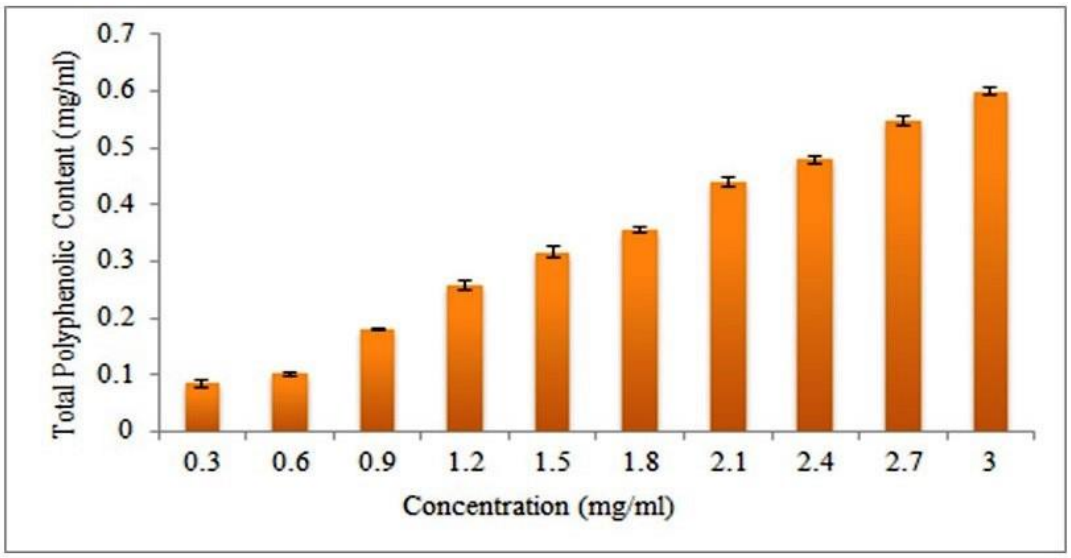


**Figure 1: Total polyphenolic content of *Cinnamomum tamala* methanolic extract of leaf**

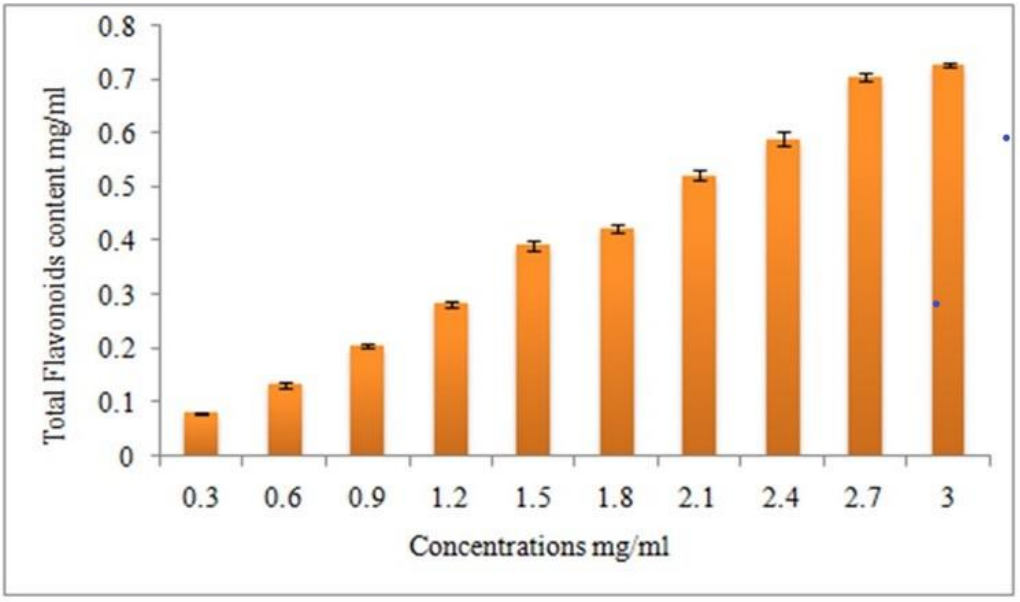


**Figure 2: Total flavonoids content of *Cinnamomum tamala* methanolic extract of leaf**

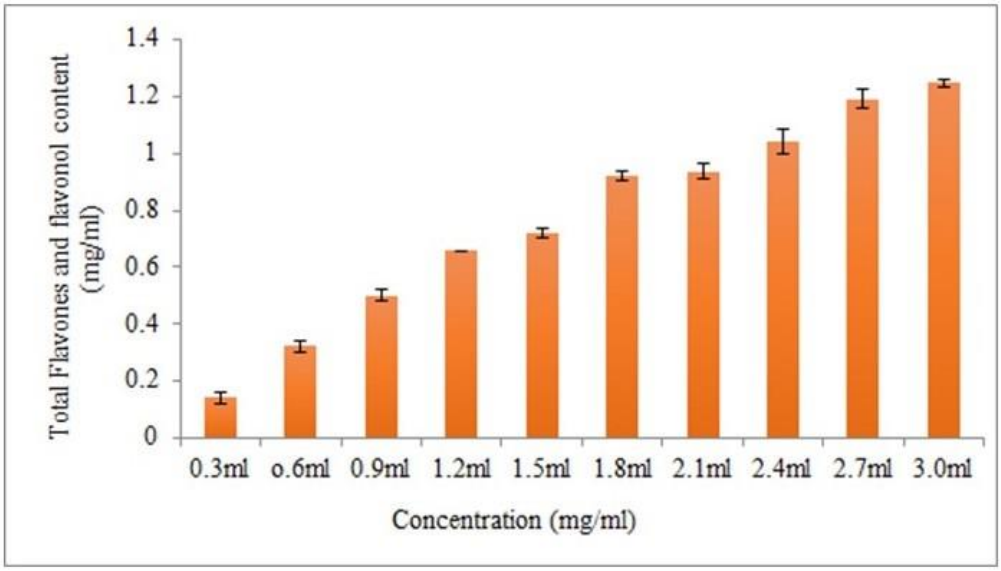


**Figure 3: Total flavones and flavonol content of *Cinnamomum tamala* methanolic extract of leaf**

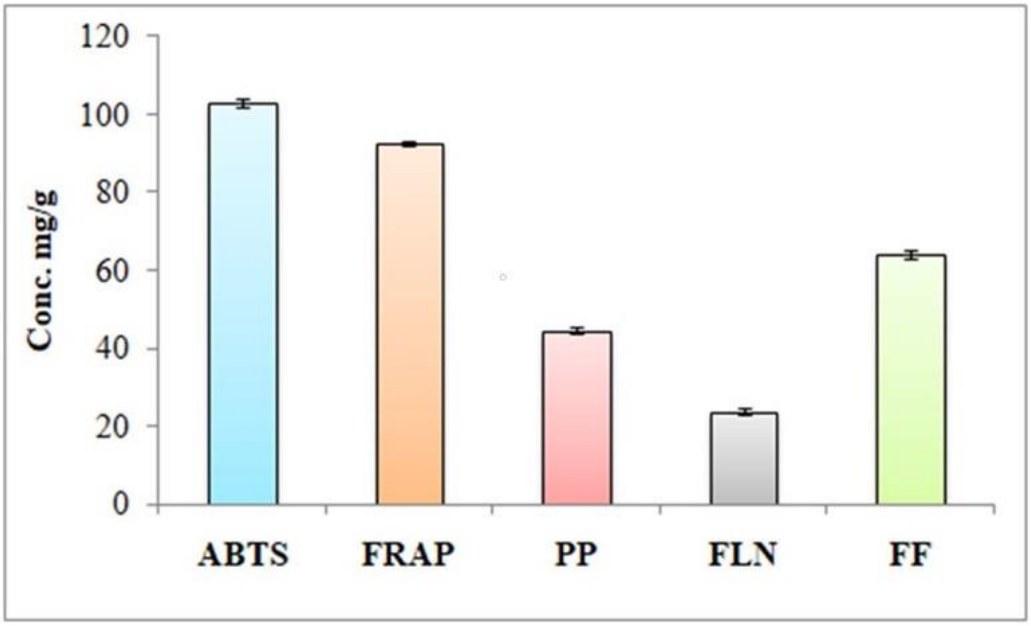


**Figure 4: Showing the different antioxidants in *Cinnamomum tamala* methanolic extract of leaf**

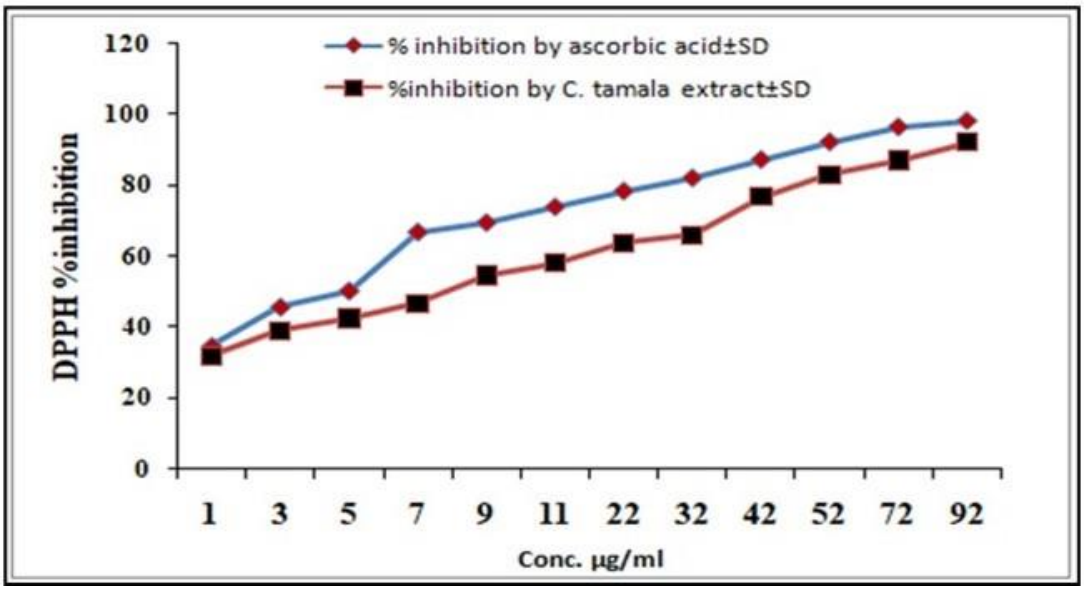


**Figure 5: Showing percentage inhibition by ascorbic acid and Methanolic leaf extract of *Cinnamomum tamala***

**Table 1: Qualitative analysis of *Cinnamomum tamala* methanolic extract of leaf.**

**Symbols:** High concentration (+++); moderate concentration (++); low concentration (+)

| Sr. No. | Phytochemicals | Test | Methanolic 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). $H_2So_4$ Test | +++ |
| 5. | Tannins | Ferric chloride Test | ++ |
| 6. | Saponins | Aqueous Test | +++ |
| 7. | Glycosides | a). $H_2So_4$ Test | +++ |
| | | b). Kellar Kilani Test | ++ |
| 8. | Proteins and Amino Acids | Millon's Test | +++ |
| 9. | Carbohydrates | Molisch Test | ++ |

**Table 2: Quantitative phytochemical evaluation of *Cinnamomum tamala* Leaf methanolic extract**

| Phytochemical (per gram of extract) | Concentration |
|---|---|
| Polyphenols | 44.5(mg GAE/g) |
| Flavonoids | 23.8 (mg QE/g |
| Flavones and flavonols | 0.67 (mg QE /g |

**Table 3: Percentage inhibition activity for ascorbic acid and *C. tamala* leaf methanolic extract.**

| Concentration (µg/ml) | % Inhibition by ascorbic acid ±SD | %Inhibition by C. tamala Methanolic leaf extract±SD |
|---|---|---|
| **1** | 34.71 ± 0.82 | 32.03 ± 0.72 |
| **3** | 45.81 ± 0.88 | 38.98 ± 0.67 |
| **5** | 50.34 ± 0.56 | 42.58 ± 0.44 |
| **7** | 66.92 ± 0.67 | 46.91 ± 1.01 |
| **9** | 69.60 ± 0.19 | 54.79 ± 0.82 |
| **11** | 74.03 ± 0.87 | 58.14± 0.39 |
| **22** | 78.51 ± 1.19 | 63.88 ± 0.91 |
| **32** | 82.22 ± 0.12 | 66.11 ± 1.62 |
| **42** | 87.39 ±0.23 | 76.87 ± 0.55 |
| **52** | 92.39 ± 0.77 | 83.12 ± 0.29 |
| **72** | 96.57 ± 0.73 | 87.04 ± 0.91 |
| **92** | 98.29± 0.89 | 92.18 ± 0.98 |