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THERAPEUTIC EFFICACY AND NUTRITIONAL POTENTIALITY OF INDIAN BAY LEAF (*CINNAMOMUM TAMALA* BUCH. - HEM.)

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ABSTRACT

C. tamala leaves are mainly used for flavouring food and spice due to clove like taste and pepper like odour. The plant leaves are widely used in pharmaceutical preparation because of therapeutic efficacy against various diseases and disorders due to presence of different phytochemicals. The leaves were analysed for ash content, moisture, crude fat, crude fibre, crude carbohydrate, crude protein and different phytochemical content. The results for percentage of ash content, moisture content, crude fibre, carbohydrate, crude fat and protein were 9.6 ± 1.12, 50.50 ± 1.0, 30.5 ± 0.6, 9.5 ± 0.5, 6.0 ± 0.5 and 8.5 ± 0.18 % respectively. The nutritive value was 143.5 ± 0.53 Kcal/ 100g. The leaf sample was assessed for quantitative and qualitative phytochemical composition. Among the phytochemicals poly phenols was highest (16.7 ± 0.7 g/100g) and flavonoid content was lowest (1.0 ± 0.31 g/100g).

Keywords: *Cinnamomum tamala*, nutritive value, phytochemical, therapeutic efficacy, physicochemical.

INTRODUCTION

Plants are used as food, medicine, fuel, fiber through the beginning of civilizations of human beings [9]. Medicinal plants are the richest bio-resource of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [36]. About 80% population of developing countries of the world depends upon medicinal plants for primary health care [8]. Medicinal plants are easily available and less expensive [11]. Most of the medicinal plant parts are used as raw drugs and they possess varied medicinal properties [31]. The review of literature revealed that considerable contribution has been made on medicinal plants [13]. Plant derived medicines are safer than the synthetic medicines, which have many adverse side effects [34]. Nasiruddin *et al* [35] reported edible wild plants are the important source of fiber, vitamins, minerals and other nutrients, which are also used for the therapy of various diseases. *Cinnamomum tamala* belonging to the family lauraceae, commonly called as Indian bay leaf. The leaf extract of this plant is used as antidiarrheal property, hypoglycemic activity and for the therapy of various kinds of ailments [41]. Present study is an attempt to quantify the nutritional value and phytochemicals composition of *Cinnamomum tamala* and study the therapeutic potentiality of phytochemicals against different diseases.

MATERIALS AND METHODS

Collection of plant material: The fresh tender leaves of *Cinnamomum tamala* were collected from Ranchi (23° 21' 0" N LR, 85° 20' 0" E L). The leaves were washed with deionised water and disinfected with 0.1% $HgCl_2$ solution for 5min and dried in shade for 15 days. The dried materials were ground to fine powder with the help of electrical grinder and sieved [22].

Physicochemical analysis

Total ash: 2g of powder was incinerated around 500-600 °C to free the sample from carbon. The percentage of ash was calculated with reference to air dried powder [40].

Determination of moisture: 2g of leaf powder was kept in pre-weighed watch glass and dried at 150°C over-night in an oven. The sample was cooled to room temperature and weighed. The weight loss in sample regarded as moisture content [38].

Crude fibre: 2g of moisture and fat free plant material was treated with 200mL of 1.25% H_2SO_4, after filtration and washing, the residue was treated with 1.25% NaOH, filtered, washed with hot distilled water and then 1% HNO_3 and again washed with hot distilled water. The residue was ignited and the ash weighed. Loss in the weight gives the weight of crude fibre [50].

Determination of Nutritive value: The nutritive values of *Cinnamomum tamala* leaf was calculated on the basis of protein, fat and carbohydrate content in the leaf sample [38].

Determination of carbohydrate: 100mg of plant sample was taken and 5mL of 2N HCl was added to it. The mixture was kept in boiling water bath for about and cooled to room temperature. Then sodium carbonate was added till effervescence ceases. The volume was made up to 100mL and centrifuged. Supernatant was collected, 0.5 and 1mL aliquots was taken for analysis. Standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 mL of the working standard; 0 serves as bland. The samples were added to each tube to make the volume 1mL. Then 4mL anthrose reagent was added and tubes heated for eight minutes in boiling water bath. Cooled rapidly and examined at 630 nm. Concentration of standard plotted on X-Axis and absorbance on Y-Axis. From the graph amount of carbohydrate present in the sample was calculated. Quantity of carbohydrate present in 100mg of the sample was calculated by following formula [17].

$$= \frac{(\text{mg of glucose})}{(\text{Volume of test sample})} \times 100$$

Determination of protein: Determination of protein and Nitrogen was done by using micro Kjeldahl method, 1g of sample of each plant taken in a Pyrex digestion tube and 30mL of conc. H_2SO_4 carefully added, then 10g potassium sulphate and 14g copper sulphate, mixture is placed on sand both on a low flame just to boil the solution, it was further heated till the solution becomes colourless and clear, allowed to cool, diluted with distilled water and transferred 800 mL Kjeldahl flask, washing the digestion flask, three or four pieces of granulated zinc and 100 mL of 40 % caustic soda were added and the flask was connected with the splash heads of the distillation apparatus. Next 25mL of 0.1 N sulphuric acids was taken in the receiving flask and distilled; it was tested for completion of reaction. The flask was removed and titrated against 0.1 N caustic soda solution using methyl red indicator for determination of nitrogen, which in turn give the protein content [21].

Determination of fat: Crude fat was determined by extracting 1g of moisture free plant material, which was treated with 200 mL of 1.25 % H_2SO_4, filtered and washed, the residue was treated with 1.25% NaOH, filtered, washed with hot distilled water and then 1% HNO_3 was added and filtered. The residue was washed again with hot distilled water. The residue was ignited and the ash was weighed. Loss in the weight gives the weight of crude fibre [50].

Phytochemical analysis: Qualitative phytochemical tests of *Cinnamomum tamala* leaf sample were conducted with previously published standards [16, 47].

Quantitative analysis of phytochemicals

Determination of alkaloid: The sample was weighed in to a 250 mL beaker and 200 mL of 10 % acetic acid in ethanol was added. Beaker was covered and allowed to stand for 4hours. Then it was filtered and the extract was concentrated on water bath to one quarter of its original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to stand till settlement of precipitate. The precipitate was collected and washed with dilute ammonium hydroxide and filtered. Alkaloid was collected as residue and weighed after complete dryness and percentage was calculated and expressed in mg/g of plant sample [17].

Determination of tannin: The analysis of tannins content in plants was performed according to AOAC method [4] and the international pharmacopoeia [46], after some modifications. 25mL of the infusion were measured into 1L conical flask, then 25mL of indigo solution and 750mL distilled deionised water (dd H_2O) were added. 0.1 N aqueous solution of $KMnO_4$ was used for titration until the blue coloured solution changed to green colour. Standard solution of Indigo carmine was prepared as following: 6 g Indigo carmine was dissolved in 500 mL of distilled deionised water (dd H_2O) by heating, after cooling 50 ml of 95 - 97 % H_2S0_4 was added, the solution was diluted to 1 L and then filtered. The blank tests by titration of a mixture of 25 mL Indigo carmine solution and 750 mL dd H_2O were carried out. The tannin content was calculated as percentage and expressed as mg/g of plant sample.

$$\text{Tannin (\%)} = \frac{(V - V_o) \times 0.004157 \times 250 \times 100}{g \times 25}$$

Where V in the volume of 0.1 N aqueous solution of $KMnO_4$ for titration of the sample (mL), V_0 is volume of 0.1 N aqueous solution of $KMnO_4$ for titration of the blank sample, ml; 0.004157 is tannins equivalent in 1 mL of 0.1 N aqueous solution of $KMnO_4$; g is mass of the sample taken for the analysis (g); 250 is the volume of volumetric flask (mL).

Determination of Saponin: 20g of each grounded sample was put into conical flask and 100 cm^3 of 20% aqueous ethanol was added. Then the flask was heated on a hot water bath for 4hr. with constant stirring at about 55 ˚C. The mixture was then filtered and the residue was again extracted with another 200 mL 20% ethanol. The combined extract was reduced to40 mL on a hot water bath at about 90 ˚C. The concentrate was transferred into a 250mL separator funnel, added 20mL diethyl-ether which as followed by vigorous shaking. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 mL of n-butanol was added. The combined n-butanol extracts were washed twice with 10mL of 5 % aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in oven, weighed and saponin content was calculated as percentage and expressed as mg/g of plant extract [39].

Determination of phenolic compounds: The amount of total phenol content, in various solvent extracts of flower was determined by Folin-Ciocalteu's reagent method [3]. 0.5mL of extract and 0.1 mL (0.5N) Folin-Ciocalteu's reagent was mixed and the mixture was incubated at room temperature for 15 minutes. Then 2.5 mL saturated sodium carbonate solution was added and further incubated for 30 min at room temperature and the absorbance was measured at 760 nm. Gallic acid was used as a positive control. Total phenol values are expressed in terms of gallic acid equivalent (mg/g of extracted compounds).

Determination of flavonoids: Flavonoids were determined as per previously published work of Boham and Kocipai – Abyazan [8]. 10 g of each sample was extracted with 100 mL of 80 % aqueous methanol repeatedly at room temperature. The whole solution was filtered through whatman filter paper #42 (125 nm). The filtrate was later transferred into crucible and evaporated into dryness over a water bath, the weight of the material and percentage quantity was calculated and expressed as mg/g of plant extract.

RESULT AND DISCUSSION

Result of physicochemical analysis of *C. tamala* leaf is represented in fig – 1. The result reveals that moisture content is high (50.5g/100g) and ash content is low (9.6g/100g) in *C. tamala* leaf. Physicochemical analysis of leaves of various medicinal plants has been reported by various workers from time to time. Indrayan *et al.* [19] reported 8.20g/100g ash, 57.90g/100g moisture and 7.20g/100g crude fibre in *A. hetrophyllus* leaves. Nasiruddin *et al.*[35] detected 2.84 ± 0.04%, 1.33 ± 0.02%, 3.50 ± 0.03% total ash, 91.60 ± 0.20%, 82.90 ± 0.74%, 85.05 ± 0.50% moisture and 0.94 ± 0.06%, 3.11 ± 0.05%, 2.41 ± 0.05% crude fibre in *Rumex crispus*, *Medicago denticulate* and *Taraxicum officinale* respectively.

The amount and composition of ash remaining after combustion of plant material varies considerably according to the part of the plant, age, treatment etc. The constituents of the ash also vary with time and from organ to organ. Ash usually represents the inorganic part of the plant [48]. Nutritionally fibre is beneficial to human boy, since it has been reported that food fibre aids absorption of trace elements in the gut [23] and reduce absorption of cholesterol [30]. Fibre aids bowel movement of gut [1]. Aravind *et al.* [5] reported, fibre of *Carica papaya* is able to bind cancer-causing toxins in the colon, keep them away from the healthy colon cells and provide synergistic protection for colon cells from free radical damage to their DNA. *C. tamala* leaf contains higher amount of ash and crude fibre (Fig-1) compare to the above studied plants thus *C. tamala* leaf contain higher amount of inorganic constituent and dietary fibres which are beneficial to health.

Primary metabolites (carbohydrates, proteins and fats etc.) are synthesized by pants which transforms into seconary metabolites (alkaloids, tennins, saponins etc). Which are used as drugs and provides nutrition [9]. Indrayan *et al.*[19] reported 19.70% carbohydrate, 5.70% protein and 2.50% crud fat in *A. hetrophyllus* leaves. Bukhsh *et al.*[9] reported 18.9 ± 4.2 %, 16.9 ± 1.1 %, 15.9± 1.3% carbohydrates and 21.87 ± 2.8 %, 16.6 ± 3.2%, 21.87 ± 4.7% crude proteins in *Carthamus oxyacantha*, *Eruca sativa* and *Plantago ovate* leaves respectively. The fat content was 6.6± 1.3% in *Eruca sativa* leaves but fat was not found in *Carthamus oxyacantha* and *Plantago ovate* leaves [9]. Nasiruddin *et al.* [35] reported 1.82 ± 0.03%, 5.99 ± 0.02%, 2.74 ± 0.01% crude protein and 0.30 ± 0.01%,

0.14 ± 0.03%, 0.21 ± 0.02% crude fat in *Rumex crispus, Medicago denticulate* and *Taraxicum officinale* respectively. Nutritional component of *C. tamala* is given in fig-2. The calculated value of carbohydrate (9.5 ± 0.5g/100g), fat (6.0 ± 0.18g/100g), and protein (8.5 ± 0.5g/100g) of *C. tamala* is higher than the above studied plants. Carbohydrate constitutes a major class of naturally occurring organic compounds that are essential for the maintenance of animal life [14]. Proteins contain amino acids utilized by the cells of the body to synthesize all the numerous proteins required for the function of the cell and also to furnish energy [42]. Due to moderate level of crude fat in the leaves, people suffering from overweight or obesity can consume in diet [35].The calculated energy is higher (143.5 ± 0.53 Kcal/100g) in *C. tamala.* Hoe and Siong[18] reported 15 Kcal, 227 Kcal, 22Kcal, 29Kcal and 34 Kcal nutritional values of indigenous fruits and vegetables *Cucumis sativus, Pangium edule, Brasssica oleraceae, Spinacia oleraceae* and *Sinapis alba* respectively. Kumar *et al.* [29] reported nutritional value of *Adhatoda vassica* leaves was106.00 Cal/ 100g. Since *C. tamala* possess high nutritional value comparing with the above studied plants and higher amount of dietary fibres. Thus leaves of *C. tamala* can be used as fodder. Result of phytochemical analysis of *C. tamala* leaves sample is presented in Fig - 3. The result revealed that polyphenols is highest (16.7 ± 0.7 g/100g) flavonoids content is lowest (1.0 ± 1.01 g/100g) among all the studied phytochemicals. Aliyu *et al*.[2] reported 0.110 ± 0.002g/100g, 0.966 ± 0.030g/100g, 1.440 ± 0.002g/100g, 7.270 ± 0.009g/100g and 2.600 ± 0.200g/100g alkaloids, 8.000 ± 0.280g/100g, 8.766 ± 0.020g/100g, 16.30 ± 0.042g/100g, 18.23 ± 0.040g/100g and 9.466 ± 0.060g/100g flavonoids, 0.533 ± 0.020g/100g, 2.500 ± 0.014g/100g, 0.900 ± 0.020g/100g, 2.320 ± 0.001 g/100g and 1.066 ± 0.020 g/100g saponins, 0.566 ± 0.010g/100g, 1.250 ± 0.009g/100g, 0.520 ± 0.200g/100g, 1.030 ± 0.014g/100g and 1.140 ± 0.001g/100g phenols in *Anchomanes difformis, Anisopus mannii, Pavetta crassipes, Stachytarpheta angustifolia* and *Vernonia blume* Des. respectively. Soladoye and Chukwuma [44] reported tannin (4.98%) in *Cissus populnea*. Khan *et al.* [25] reported tannin content was 15.75% in *M. rubicaulis*, 14.16%, *W. fruticosa*, 13.4% in *C. grata*, 12.33% in *V. cotinifolium*, 11.2% in *E. hirta*, 10.56% in *B. Papyrifera* and 10.2% in *P. harmala*. Flavonoids prevent injury by direct scavenging of free radicals. The hydroxyl group of the flavonoid directly stabilize the reactive oxygen free radical and other free radicals because of the high reactivity of the hydroxyl group of the flavonoids and inactive the free radicals [26]. As antioxidant by nature flavonoids inhibits carcinogenesis [45]. Flavonoids act as antiviral agent by inhibiting activity of reverse transcriptase, RNA-directed DNA polymerase [37], anti integrase and antiprotease [33]. Flavonoids diminish the activities of cyclooxygenase and lipoxygenase and inhibit inhibits inflammation. Saponin kills pathogenic protozoa by forming complexes with sterols in the protozoa membrane surface due to which the membranes become impaired and eventually disintegrate [49]. Mengoni *et al*. [32] reported saponin inhabits HIV-I virus replication by inhibiting HIV-I protease activity. Saponin acts as immune modulator by induce production of interlukins and interferons in human body [24]. Tannin induces hypo cholesterolemic effect by blocking the propagation of free radicals and induces oxidation of LDL cholesterol [6].Chang [12] reported tannin inhibits 3-hydroxy-3methyl CoA reductase, necessary to cholesterol biosynthesis. Alkaloids possess anti-oxidizing effects, thus reduces the nitrate generation which is useful for protein synthesis, suppresses the transfer of sucrose from stomach to small intestine and steroids enhance intestinal absorption of sodium ions and water. Isaac and Chinwe [20] reported that alkaloids are responsible for the antibacterial activity. Plant phenolics are potent inhibitors of a number of growth factor binding and signalling pathways implicated in cancer. Thus plant phenols inhibit epidermal growth factor receptor action and reduce the invasive potential of cancer cells [28, 27].*Cinnamomum tamala* possess antibacterial activity due to the presence of certain phenolic compound such as cinnamic aldehyde and such as eugenol and cinnamic acid [7]. An important characteristic of leaf extract and their components of is their hydrophobicity, which enables them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structure and rendering them more permeable [43].

CONCLUSION

The present study suggests that *C. tamala* leaf, which contains higher amount of primary metabolites (fats, carbohydrates, and proteins) and secondary metabolites (flavonoids, saponin, tannins, alkaloids and polyphenols) can be used as fodder as well as therapeutic agent against the above mentioned disease.

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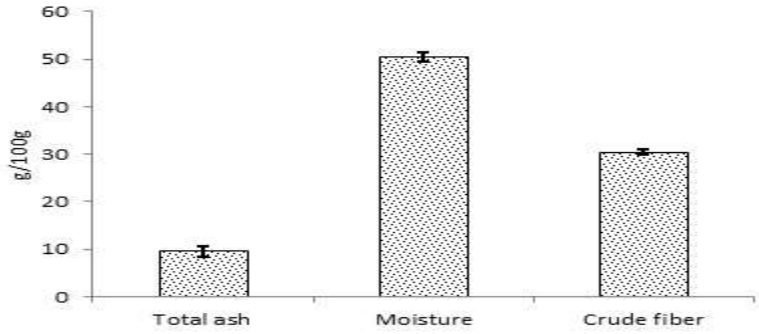

Fig 1: Physicochemical composition of *C. tamala* leaves in g/100g (M ± SD; n=3).

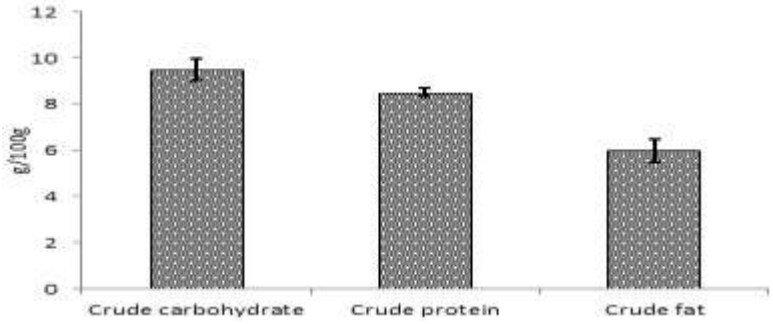

Fig 2: Fat, protein and carbohydrate content of *C. tamala* leaves in g/100g (M ± SD; n=3).

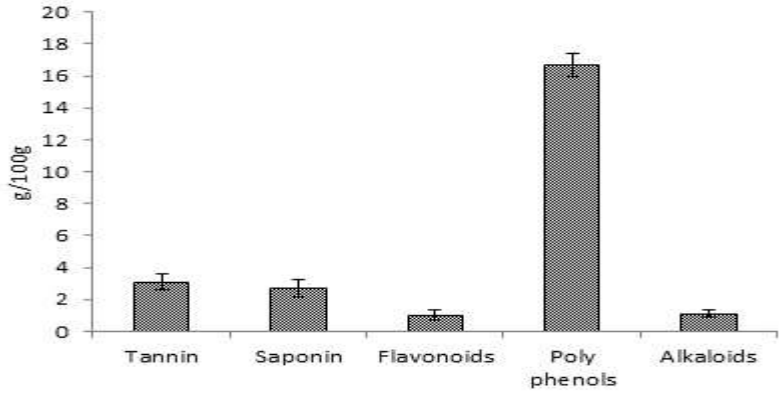

Fig 3: phytochemical composition of *C. tamala* leaves in g/100g (M ± SD; n=3).

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