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Evaluation of protein content and antioxidant potential of *Terminalia catappa*

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ABSTRACT

This study is carried out to evaluate the nutrient contents and possibility of promoting *Terminalia catappa* usage as human food or animal feed. Quantitative information regarding protein content of extracts was determined by Bradford and FC method while the antioxidant potential of extract was evaluated using DPPH free radical method. Furthermore, ultracentrifugation and SDS gel electrophoresis was used to get more detailed description of protein content. Various parts of *T.catappa* can be used as food supplement. It can also be used raw or roasted to improve the food nutrient content as it is rich in proteins.

KEY WORDS: *Terminalia catappa*, Bradford assay, antioxidant, DPPH assay.

INTRODUCTION

With the growing demand for protein, interest in plant proteins is also rising, multiple factors are contributing to this growth, such as food safety concerns, ethics, environmental concerns, rise in food intolerances, allergies and "free-from" foods, sustainability, increased accessibility of vegetarian and vegan foods and the adoption of proactive approaches to health and wellbeing by consumers. The quest for dairy-free and soy-free products is also driving the rise in alternative sources of protein such as pea, pulse, nut and seed, quinoa, rice,

hemp, potato, and oat proteins among others. Research indicates that replacing some animal-based protein with plant-based protein sources may help decrease the risk of developing chronic diseases such as heart disease, diabetes and some cancers. Plant-based foods contain important antioxidants, vitamins, and minerals that benefit overall health. Additionally, the fiber in plant proteins lend to increased satiety and improved gut health. Plant-based proteins also have a lower environmental impact as more greenhouse gasses are emitted with the production of animal-based proteins[1].

Terminalia catappa is a shade and salt tolerant street tree, which was primarily used as an ornamental plant [2]. They are most commonly found on tropical and subtropical beaches [3]. In India, it is known as Malabar almond, Indian almond and tropical almond. They are widely seen throughout the warmer parts of India and also in other countries like Australia, Srilanka, Malaysia, Pakistan and many other South Asian countries[4].The plant grows mostly in well aerated, freely drained, sandy soils and they can withstand strong winds, the salt sprays. By providing a wide range of non-wood products and services, it plays a vital role in coastal communities [5]. In the traditional Indian system of medicine, the Ayurveda and various folk system of medicine, *T. catappa* possess several medicinal properties. It is very rich in phytochemicals and a good source of natural antioxidants [6]. Parts of the tree, such as the leaves and fruit, are astringent. The red leaves act as a vermifuge, while the sap of young leaves, cooked with oil from the kernel, is used to treat leprosy. Leaves, bark and fruit are used to treat yaws. The bark and root bark are useful for bilious fever, diarrhoea, thrush, and as a remedy for sores and abscesses. The kernel of the fruit mixed with beeswax stops putrid exudation and bloody faeces. It is recommended as a mild laxative and a galactagogue for women, but too frequent use causes diarrhoea. Keeping in view, the benefits of plant proteins on long-term health and chronic diseases, we have studied the protein value and antioxidant potential of *T.catappa* in this work.

MATERIALS AND METHODS:

Collection of plant sample

The fully mature *Terminalia catappa* (Leaves, fruits, seeds & bark) were collected in May-June 2022 from Freedom Park, Bengaluru, India and the plant was authenticated by Prof. Dr Suresh Kumar, Department of Botany, Maharani Cluster University, Bengaluru.

Processing of plant sample

The leaves, fruits, seeds and bark of *Terminalia catappa* were properly washed in tap water and then rinsed in distilled water. The rinsed plant sample were shade dried for 2-3 weeks and powdered using electrical blender. The powders were stored in air tight glass containers, protected from Sunlight until required for analysis.

Solvent extraction

About 1 gm of each powdered plant material was taken in conical flask with 10ml of methanol. The solution is kept in shaker incubator for constant shaking to about 3hours at the

speed of 85rpm and at 37^0 C temperatures. The obtained extracts were filtered by using whatman filter paper, transfer the filtrate into separate test tubes and residues into petri dishes. The filtrate was stored at 40^0 C until further use and allow the residues in petri dishes to dry until methanol gets evaporated. Each extracts was resuspended in the solvent and used for qualitative and quantitative analysis of phytochemicals.

Quantitative estimation of proteins

Bradford protein assay: It is a quick and fairly sensitive method for measuring the amount of proteins. Determination of microgram quantities of protein in the Bradford Coomassie brilliant blue assay is accomplished by measurement of absorbance at 590 nm. Dilute standard sample in different concentration with extraction buffer. Prepare a 0.1 mg/ml stock solution of the standard, bovine serum albumin (BSA). Add 1.0ml of dye reagent to sample incubate for 30 minutes. Take the absorbance at 595 nm with blank, set as zero in spectrophotometer [7].

Lowry Protein assay

This assay is based on the Biuret reaction with additional steps and reagents to increase the sensitivity of detection. In the Biuret reaction, copper interacts with four nitrogen atoms of peptides to form a cuprous complex. Lowry adds phosphomolybdic/ phosphotungstic acid also known as Folin-Ciocalteu reagent. This reagent interacts with the cuprous ions and the side chains of tyrosine, tryptophan, and cysteine to produce a blue-green colour that can be detected between 650 nm and 750 nm [8]. Prepare 100mg/ml stock solution of the standard BSA. Prepare the working solution from standard BSA of 200µg/ml of different concentrations and made final volume 1.0ml with distilled water. Add copper reagent of 5.0ml to standards and samples. After adding copper reagent incubate for 10 min at room temperature. After incubation, add 0.6ml of FC reagent to standards and samples. Read the absorbance at 660 nm using “blank” set as zero in calorimeter.

Determination of size of protein

SDS–PAGE (Polyacrylamide Gel Electrophoresis of Proteins): is a technique for separating proteins based on their ability to move within an electrical current, which is used with a reducing agent and heat to dissociate the protein. SDS-Polyacrylamide complexes form and migrate through the gels according to the size of the polypeptide. By using markers of known molecular weight, the molecular weight of the polypeptide chain(s) can be estimated. Assemble the glass plates according to the biorad instructions. Determine the volume of the gel mold in an Erlenmeyer flask or disposable plastic tube prepare the resolving gel using the appropriate volume of solution containing the desired concentration of acrylamide using the values given. Polymerization will be as soon as the TEMED has been added. Without delay, swirl the mixture rapidly and proceed. Pour the acrylamide solution into the gap between the glass plates. Leave sufficient space for the stacking gel. Use a Pasteur pipette to overlay the acrylamide solution carefully with 0.1% of SDS or isobutanol. Place the gel in a vertical position at room temperature. After polymerization is complete, pour off the overlay and wash the top of the gel

several times with deionized water to remove any unpolymerized acrylamide. Drain as much fluid as possible from the top of the gel, and then remove any remaining water with the edge of a paper towel. In a disposable plastic tube, prepare the stacking gel using the appropriate volume of solution containing the desired concentration of acrylamide. Polymerization will be as soon as the TEMED as been added. Without delay, swirl the mixture rapidly and proceed. Pour the stacking gel solution directly onto the surface of the polymerized resolving gel. Immediately insert a clean Teflon comb into the stacking gel solution. Avoid trapping air bubbles. Add more stacking gel solution to fill the spaces of the comb completely. Place the gel in a vertical position at room temperature. Attached the electrophoresis apparatus to an electric power supply. apply voltage to the gel after the dye front as moved into the resolving gel, increasing the voltage and run the gel until the bromophenol blue reaches the bottom of the resolving gel then turn off the power supply. Remove the glass plates from the electrophoresis and place on the paper towel; mark the orientation of the gel by cutting a corner from the bottom of the gel. After that, the gel can be fixed, stained with coomassie brilliant blue and kept for overnight later destain the gel and view under fluorography [9].

DPPH antioxidant assay

DPPH is a stable free radical chemical with purple color that absorbs at 517 nm. About 1 mL of test solution of ethanol, methanol and chloroform extract were dissolved in equivalent amount of DPPH solution (0.1 mM). The increase in DPPH absorbance of tested samples was measured after 20 min incubation at room temperature, by taking the absorbance. Standard ascorbic acid (1 mM) showed the maximum absorbance of 90.36 ± 1.05 µg/mL, and was taken as a reference solution in this DPPH antioxidant assay. The formula used to calculate the percent (%) inhibition was DPPH percentage[10]

(%) inhibition = ((AB-AA)/AB)×100. where, A_B denotes the absorbance of DPPH radical + methanol; A_A represents the absorbance of DPPH radical + sample extract/standard.

Results and Discussion

The results of protein assay indicates more protein in exocarp and mesocarp, fruits, bark, and leaves(Fig.1,2)

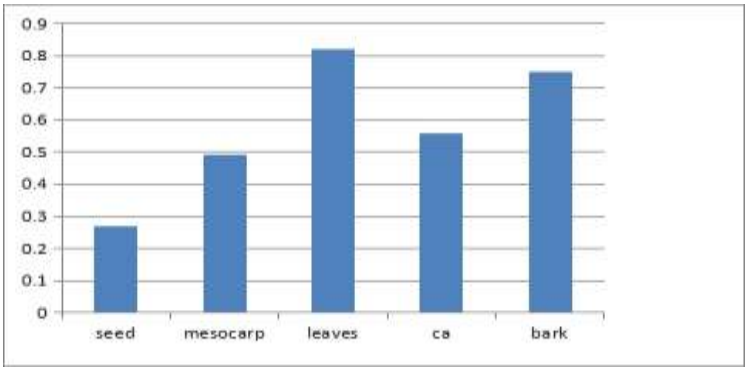

Fig.1: Protein estimation by FC method

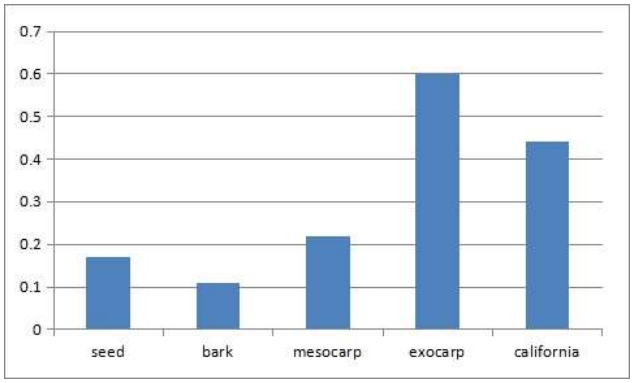

Fig.2: Protein estimation by Bradford method

SDS-Polyacrylamide complexes form and migrate through the gels according to the size of the polypeptide. By using markers of known molecular weight such as BSA (66kDa) and Lysozyme (14kDA), the estimated molecular weight of protein in crude methonolic extract of *T. catappa* seed is approximately found to be 63kDa.

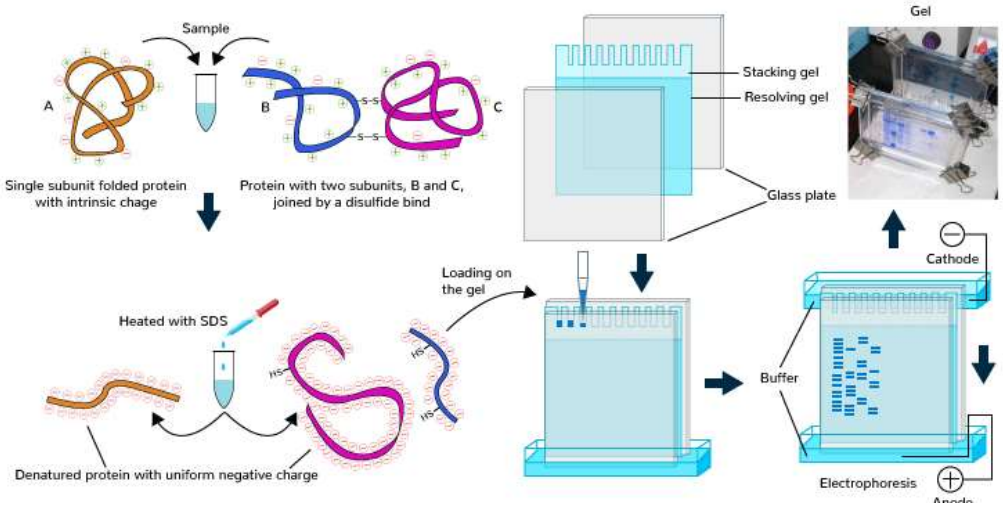

Fig.3: SDS-PAGE for separation of *T. catappa* proteins.

SDS PAGE is used for characterization size of the protein using molecular markers such as BSA and Lysozyme. The molecular weights of proteins of plant extracts are found to be

approximately to 66kDa. Chemical composition analyses indicated that the seed has a good nutritional value with typically low moisture, high proteins and lipids.

Fig.4: SDS-PAGE A= Markers (BSA and Lysozyme) B=Seed

It can be observed from Table.1 that the antioxidant activity of *T.catappa* methanol extract of stem and bark showed 0.550 absorbance at 517 nm with the percentage of antioxidant as 43.415% and ethanol extract showing absorbance of 0.461 at 517 nm with the percentage of antioxidant as 57.783% with DPPH(table.1).

Table.1: Antioxidant activity of T.catappa seeds with DPPH assay

Extracts	Absorbance	% of antioxidant activity
Ethanol	0.550	43.41
Chloroform	0.461	57.78
Methanol	0.552	60.72

CONCLUSION

In many developing countries animal protein constitutes a very less percentage, so dietary sources becomes a plant source, as a choice it is an alternate arrangement as cheaper and feasible food. The nutritional value of *Terminalia catappa* seed as a source of dietary protein was investigated that the crude protein was high with essential amino acid which is highly digestible, support growth, positive nitrogen balance thus protein.. In addition, heavy metals were only present at traces level, indicating that *Terminalia* plants would also be safe for medicinal uses.

CONFLICT OF INTEREST: The authors declare that they have no conflicts of interest to disclose.

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