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Phytochemical and Nutritional Potential of Tropical Almond Seed and its Antifungal Activity against Candida Albicans

Bindu. P.K[1], Aswathy. K.S[2], Neethi Raj P[3], Meritta Joseph[4]
[1]*Department of Postgraduate studies and Research in Botany, Sanatana Dharma College, Alappuzha, University of Kerala*

Abstract: ***Introduction: Nuts play an important role in the diet of several people worldwide. They will act as a supplement of nutrients. The goal of the present study was to evaluate the nutritional relevance of Terminalia catappa Linn (tropical almond) grown in the coastal area of Alappuzha and to screen secondary metabolites. The study also assessed the antioxidant activity as well as the antifungal activity of methanol extracted seed against Candida albicans. Method: For the nutritional analysis, the powdered sample was tested for its nutritional value by standard protocol. Phytochemical screening was done on methanol extract of tropical almond and the antioxidant activity was detected by DPPH radical scavenging assay and antifungal activity was detected by determining the zone of inhibition on plate method. Results: The results showed that tropical almond contained a remarkable amount of macronutrients and also vitamins. A wide variety of phytochemical was also detected in methanol extract of tropical almond. The methanol extract of seed showed remarkable inhibition of the fungal growth against tested fungal species. Considering the antioxidant activity of the seed, it was well noted that when the concentration of the sample increased the antioxidant activity also shows an upward trend. Conclusion: Tropical almond is shown to be an important source of nutrients and secondary metabolites. The antioxidant activity and antifungal activity was also detected in the tropical almond seed. Hence, this seed can be used to discover bioactive natural products that may help as a lead in the development of new pharmaceutical application mainly for antifungal drug and cream based formulation against antibiotic resistant fungal strains.***
Keywords: Terminalia catappa, Tropical almonds, Candida albicans, antifungal activity and antioxidant activity

I. INTRODUCTION

The pathological microorganism is the agents which are responsible for the increased diseases and death in the rural and urban area. Different types of commercial antibiotics are available for treatment but the emergence of antibiotic resistant strains is a serious annoyance in the health care sector. To overcome this problem, the treatment and control of diseases by the use of medicinal plants will continue to play significant roles. For this, a systemic study of medicinal plants is very important to establish a treatment modality. Terminalia catappa Linn (Tropical almond) is innate to Southeast Asia. It is a large tree that grows very well in tropical and subtropical climatic conditions, and is also well recognized as a herb in Ayurveda. The juice of T. catappa fresh leaves is used in the preparation of medical lotion for leprosy and scabies, and it is advanced for stomachache and headache. Several studies report that leaves of T. catappa are rich sources of several medically important polyphenolic compounds like tannin and also has the capacity to act against various pathogenic organisms (*Akharaiyi* et al., 2011). Different extracts of T. catappa show good antimicrobial activity against gram positive and gram negative microorganisms. The chloroform root extracts show antimicrobial activity against Escherichia coli and Staphylococcus aureus and aqueous and methanolic extracts of the T. catappa leaves show multiple activities against several pathogenic bacteria like Staphylococcus epidermidis, Staphylococcus subflava, Proteus mirabilis (Nair and Chanda et al., 2008). Numerous pharmacological investigations have confirmed these plants parts ability to exhibit antimicrobial, antioxidant and anticancer activities, which support its traditional uses.

In Taiwan, the nut of tropical almond is considered to have cherished by many people in the rural areas of Southern Nigeria. However, there is information abound on the nutritional content of different nuts such as groundnut, cashew nut, walnut etc., only limited information is available on the nutritional and medicinal effect of tropical almond. Nagappa et al. (2003) studied the antidiabetic activity of T. catappa fruits on alloxan-induced diabetic rats. Foods have an effect on the immune system, hormones, brain and heart health, they truly do act like medicine once consumed. And it is necessary to study the nutritional importance of T. catappa grown in *coastal areas* and its pharmacological investigation to validate the immense potential of this plant for the treatment of numerous diseases.

II. MATERIALS AND METHODS

A. *Sample Collection*

The nuts used for the study were collected in the field of the coastal area of Alappuzha district, lies in the coastal lowland division of Kerala. The city is located between 9.50° North Latitude and 76.33° East Longitude. The fruits were washed with water and air dried. The nuts were shelled manually and screened to remove bad seeds. Prior to the analysis they were sun-dried and sealed in plastic bags. The nuts were ground to fine powder and dried in a desiccator under vacuum at room temperature to get constant weight.

B. *Nutrient Analysis*

These analyses were conducted to determining the amounts of carbohydrate, protein, fat, vitamin A, vitamin C, folic acid, calcium and moisture present in the sample. Three replicate aliquots of the almond were tested for carbohydrate by the method described by Hodge & Hofreiter (1962). The protein content of the material was estimated following Lowry *et al.* (1951). Fat content was determined by the method introduced by Cox & Nielson (2003). Vitamin A was estimated by the method of Bayfield and Cole (1980). For vitamin C quantification was done by the method described by Bhuvaneswari *et al.* (2015). Folic acid, calcium and moisture content were also tested described by Etienne *et al.*, (2015), Horwitz *et al.*, (1975) and Hart *et al.*, (1961) respectively

C. *Phytochemical Analysis*

The methanol extract of dried samples were further screened for their phytochemical analysis based on the standard procedure in order to detect the presence of Alkaloids, Carbohydrates, Flavonoids, Phenols, Saponins, Tannin, Terpenoids, Quinones and Proteins according to the method described by Prabhavathi *et al.* (2016).

D. *Antioxidant Activity*

The DPPH radical scavenging activity of tropical almond extract was measured by the method described by Brand-William *et al.* (1995). The absorbance of the mixture was measured at 517 nm on a spectrophotometer. Quercetin was used as a positive reference standard. The DPPH free radical scavenging activity was calculated and the results were expressed as percentage inhibition of the DPPH free radical.

E. *Antimicrobial Activity*

Determination of the zone of inhibition method

In vitro antifungal activity of methanol extract of tropical almond were tested against *Candida albicans*. For the determination of zone of inhibition, Mueller-Hinton sterile agar plate was seeded with indicator bacterial strain (*Candida albicans*) (10^8 cfu/mL) and incubated for 3 hours at 37°C. Control experiment were carried out under similar condition with fluconazole for antifungal activity as a standard drug. The zone of growth inhibition around the spotted sample was determined after 18 to 24 hours of incubation at 37°C. The sensitivity of fungal sample to the extract was determined by measuring the size of inhibitory zone including the standard drug on the agar surface and the value <8mm was considered as not active against microorganisms.

III. RESULTS

A. *Preliminary Nutrient Analysis*

The carbohydrate concentration in the tropical almond seeds was found to be 162mg/g, in the case protein it was 208.60mg/g. Fat content was found to be 14.5% and the concentration of vitamin C was 0.832mg/g and the amount of folic acid was 0.82mg/g. For calcium content, it was 2.29mg/g. The moisture content of nuts was found to be 2.2%. Detailed values of nutrients were given in table 1.

Table 1: Nutrients content in methanol extract of tropical almond seed (*Terminalia catappa, L*)

Nutrients	Concentration (%)
Carbohydrate	16.2
Protein	20.86
Fat	14.5
Vitamin A	0.64
Vitamin C	0.0832
Folic acid	0.082
Calcium	0.229
Moisture	2.2

B. Phytochemical Analysis

It was found that methanol extract of tropical almond contained carbohydrate, phytosterols, flavonoids, tannins and phenolic compounds.

C. Antioxidant Activity

DPPH radical scavenging assay is commonly used to evaluate the antioxidant activity of a compound for a short period. The highest percentage of inhibition was detected when the concentration increase. The real activities of the sample was compared by the amount of antioxidant required to scavenge 50% of DPPH free radicle (IC_{50}). Lower the IC_{50} signifies higher antioxidant activity, here the methanol extract of tropical almond shows lowest IC_{50} ie, 11.46.

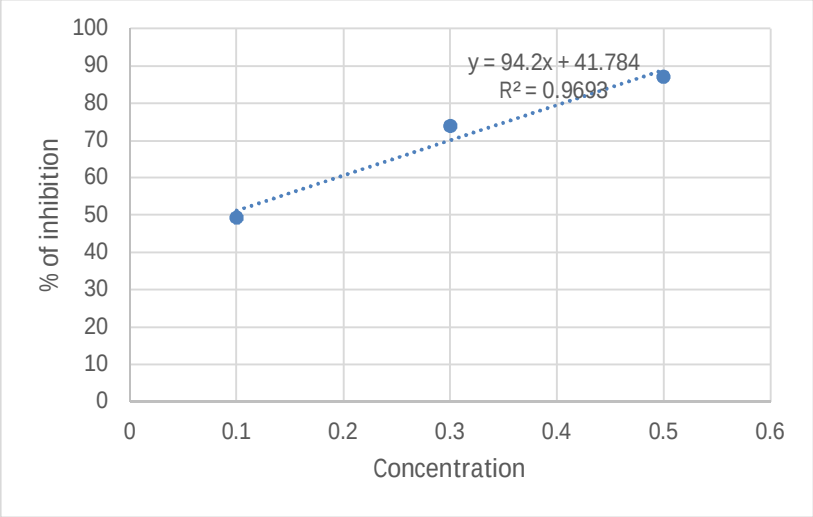

Fig 1. Antioxidant activity curve of *T.catappa* phytochemicals

D. Antifungal activity

The antifungal activity of methanol extract of tropical almond was tested against *Candida albicans* in terms of zone inhibition of fungal growth. The results of antifungal activity were presented in Figure 2.

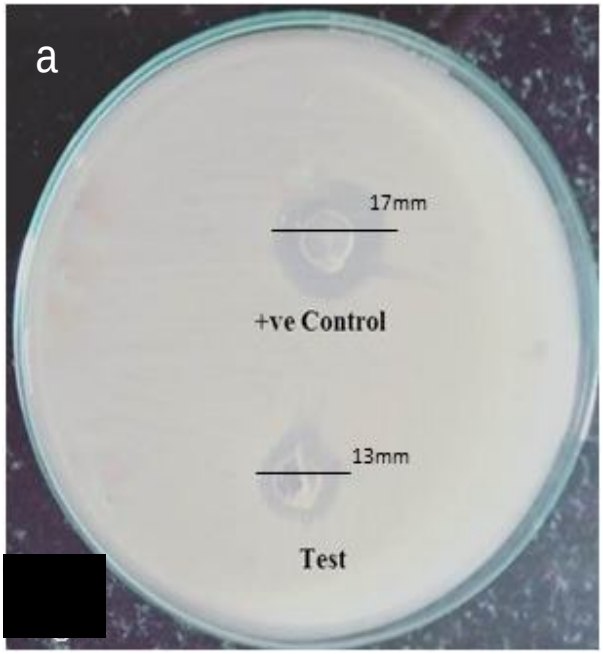

Fig 2. Antifungal activity of extract aganist C.albicans

IV. DISCUSSION

The proximate nutrients analysis shows that *Terminalia catappa L* had a rich source of nutrients. Important macronutrients like carbohydrate and protein and fat are present in these seeds in adequate quantity. Minerals are very important in the diet because they act as cofactors for various metabolic functions. Among the nutrients analyzed, the protein was detected in high amount (20.86%) followed by carbohydrate (16.2%) and fat (14.5%). Carbohydrate is considered as an important provision of energy and according to FAO (1997), at least 55% of total energy must be obtained from carbohydrate. Diet with high carbohydrate content may help to reduce obesity by preventing overconsumption of energy. Calcium is an important mineral for bone formation and neurological functions and this mineral was found to be present in significant level in methanol extract of tropical almond. The proximate analysis also shows that important vitamins like vitamin A, vitamin C and folic acid (vitamin B9) were also present in high concentrations in the tropical almond seeds. Among them, vitamin A was detected in the highest amount which was 6.40 mg/g followed by vitamin C and folic acid, 0.832 mg/g and 0.82s mg/g respectively. Vitamin A deficiency (VAD) is currently a major problem in developing countries.

Vitamin A deficiency is mainly detected in pre-school children and pregnant women in low income countries. Vitamin C is an essential nutrient involved in the enzymatic production of certain neurotransmitters. This vitamin is involved in the repair tissues and required for better functioning of several enzymes. Vitamin C also functions as an antioxidant and it is important for immune system functioning. Vitamin C deficiency in the body leads to scurvy, a disease resulting from the breakdown of collagen. Folic acid is another micronutrient which is essential for body functioning. This micronutrient is required to make DNA and RNA and for the metabolism of amino acids which are required for the cell division. Dietary surveys in India show that people having cereal based diet consume only about 75µg of folic acid (Guidelines on Food Fortification with Micronutrients, 2006).

Marques *et al.* (2010) studied the nutritional status of *T. catappa* fruit, in his study the vitamin C content was very low. However, in this study, we could found 0.832mg/g vitamin C in the nut.

Our result also agrees with the findings of Mark and Ukhan (2006) that tropical almond is a good energy food and its water content is very low when compared to other nuts. Hence this nut will have good storage properties. The results of this present study inform the potential value of the tropical almond nut. But the bioavailability of the essential nutrients studied should be conducted in order to provide the complete picture of the nutritional significance of tropical almond nut.

Most of the research done so far has mainly concentrated on biological and phytochemical screening of leaves, bark and fruit extract. In some countries, *T.catappa* seed is eaten as a snack item. Even though the seed is edible, but only limited information is available on the nutritional value of *T.catappa* seeds and no previous studies have investigated the antioxidant and antifungal activity of *T.catappa* seed. In this study, the antioxidant activity of methanol extract of tropical almond showed significant antioxidant activity when the concentration increased. Plants are rich source of secondary metabolite this plethora of compounds give plants antioxidant and antimicrobial activity. In this work, we detected carbohydrate, phytosterols, flavonoids, tannins and phenolic compounds in *T.catappa* nuts. `

This can be one of the reasons for the increased antifungal activity of nuts against *Candida albicans*. In previous reports, the methylene chloride extract and methanol extract of *T. catappa* showed antifungal activity against different fungal species (Goun *et al*., 2003; Parimala *et al*., 2015). The same result was observed for tree bark also. However, no reports are available on the antifungal activity of methanol extract of *T. catappa* seed on the fungal strain. In this study the methanol extract of seed showed significant antifungal activity against *Candida albicans* and the growth of inhibition zone measured as 13 mm (Fig:1) against fungal strain. These two aforementioned characteristics of tropical almond nut confirm the possibility of using this nut for various infectious treatment.

V. CONCLUSION

The current results from the study show that *Terminalia catappa* seeds contain a considerable amount of nutrients when compared with other widely used nuts. And also confirm that this tropical almond is a good energy food and due to less water content they have good storage properties. The methanol extract of nut was found to be active against fungal strain *Candida albicans*, so present study highlights the potential discovery of new natural bioactive compounds from tropical nuts for the formulation of antifungal drugs and antifungal creams. However, further studies are needed to better evaluate the potential efficiency of the crude extract as the antimicrobial agents as well as clinical trials are needed for the product development to support the use of *T. catappa* seeds for future generations.

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VII. CONFLICT OF INTEREST

No conflict of interest

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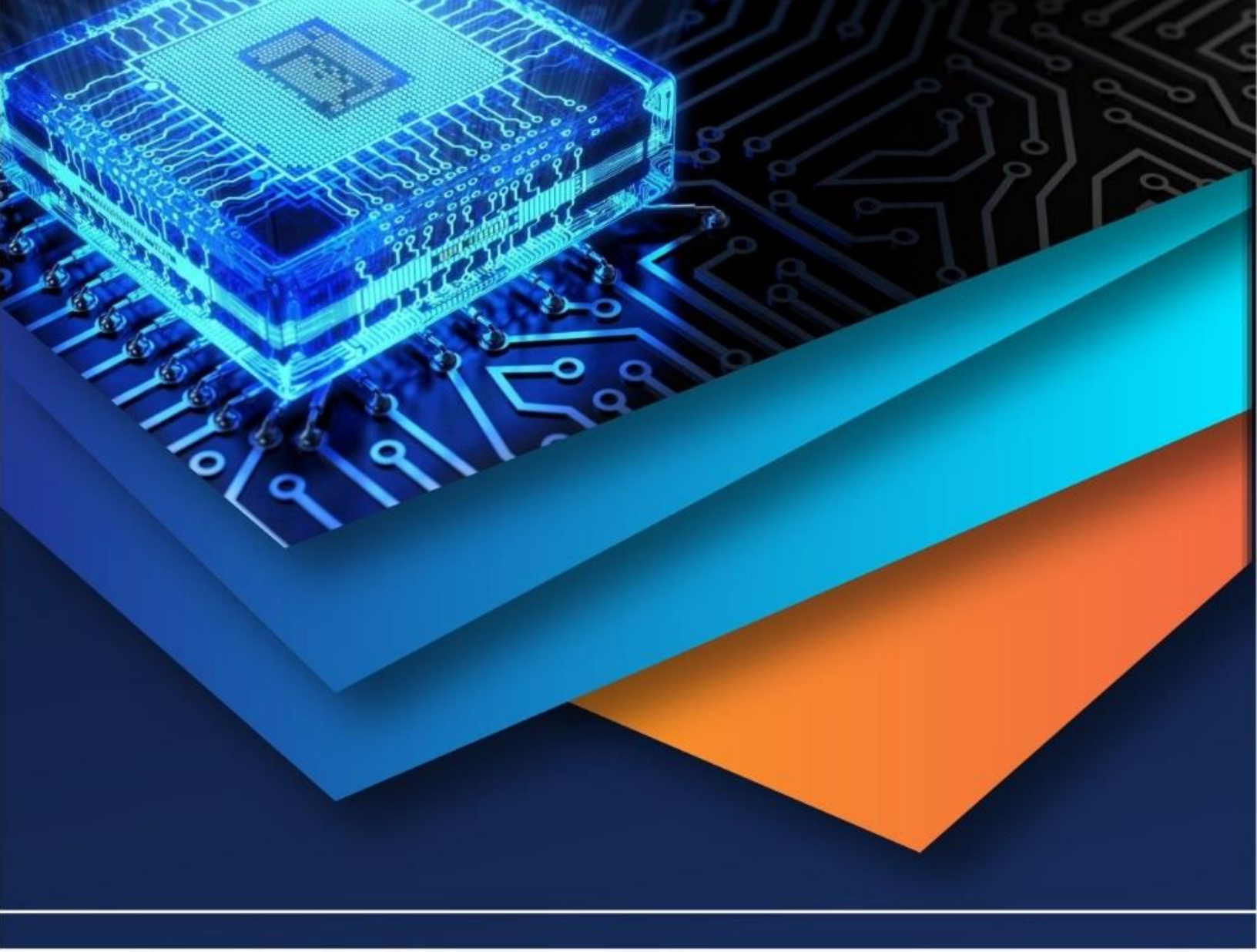

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