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Total Phenolic, Antioxidant and Antimicrobial properties of *Manihot esculenta* leaves

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Abstract

Plants with various medicinal properties have been source of attraction for many scientists all over the world since thousands of years. Millions of plants have been studied extensively since ancient times for various antioxidants and their possible medicinal uses in various disease conditions in human beings. Phenolic compounds are widely distributed in the plant kingdom. Presence of phenols is considered to be potentially toxic to the growth and development of pathogen. Antioxidants are substances neutralizing the free radicals and prevent them from causing cell damage and also inhibit or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress. Anti-microbial agents are undeniably one of the most important therapeutic discoveries of the 20th century. Cassava (*Manihot esculenta*) is a perennial shrub of the Euphorbiaceous family, cultivated mainly for its fleshy tuberous roots, and is one of the most important sources of food energy and industrial raw materials in tropical and subtropical regions. The present study mainly focuses on the total phenolic, antioxidant and antimicrobial properties of *Manihot esculenta* leaves. The results showed that the phenolic compounds present in the leaves may contributed to the antioxidant and antimicrobial activity of cassava.

Keywords: Total Phenol, Antioxidant, Antimicrobial and *Manihot esculenta*

1. Introduction

The plants are the natural reservoir of medicinal agents almost free from the side effects normally caused by synthetic chemicals. The World Health Organization estimates that herbal medicine is still the main stay of about 75-80% of the world population, mainly in the developing countries for primary health care because of better cultural acceptability with the human body, and lesser side-effects. The plant-based traditional medicine system

continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care. Polyphenols are widely distributed and important class of plant secondary metabolites, which possess aromatic ring with one or more hydroxyl substituent. Phenolic compounds are mostly water soluble since they are most frequently occurring in combination with sugars as glycosides (Harborne, 1998). Polyphenols are divided into many classes. Free phenols and phenolic acid are considered together in one class because free phenols are relatively rare in plants. Plant polyphenols are very important for growth development and play key role in defense against microbial activities, and infections. Antioxidants significantly decrease the adverse effect of reactive species and at the same time antioxidant therapy has great impact in the treatment of many other diseases (Bahekar *et al.,* 2013).

2. Materials and Methods

Sample collection: The Fresh leaves of Cassava (*Manihot esculenta)* were collected from our **own field** and preserved for the future use.

Preparation of plant extract: The collected leaves were cleaned and cut into small pieces, about 50gms of chopped leaves were taken for the extraction. Two different solvents were used for the extraction; acetone and methanol for 2-3 days in rotator shaker. All the extracts were filtered and stored in brown bottles at 4°C.

Total phenolic Assay: Total phenolic content was quantified by using Folin- Ciocalteu's method with some modifications.

Antioxidant Activity

Ascorbic acid: This assay was carried out by the method of sadasivam *et al.,* (1997).

Reducing Power: The reducing power was determined according to the method of Singh *et al.,* (2002).

β-carotene: This assay was carried out by the method of Anderson *et al.,* (2013).

Antimicrobial activity: Five species of bacteria's were used to test the antimicrobial activity of different cassava leaf extracts, among that, four gram negative (*Escherichia coli, Klebsiella pnuemoniae, Pseudomonas aeruginosa & Serratia marcescens)* and one gram positive (*Staphylococcus aureus*) were obtained from Kongunadu arts and science college, Coimbatore.

Preparation of bacterial plates: The plates were prepared and the bacterial cultures were grown in Nutrient agar media. The inoculums were prepared with nutrient broth and incubated on a rotary shaker at 37°C for 24 hours.

Disc diffusion method: The antibacterial activity of standard agar disc diffusion method was adopted from Appendini *et al*., 2002 with some modifications. Spectinomycin (100mcg/disc) was used as a positive control with the tested bacteria. The diameters of the inhibition zone (IZs) were measured in millimeters.

3. Results and Discussion

Recently scientific interest in medicinal plants has burgeoned due to the increased efficiency of plant derived drugs and raising concern about the side effects of modern medicine. Total phenol content was found to be high in methanol extract than the acetone (Table 1). Gallic acid was used as standard for determine total phenol content. Ascorbic acid (Vitamin C) was very low in acetone extract. High content was found to be in methanol extract of cassava leaves (Table 1). Our results suggested that the total phenolic and ascorbic content was high in methanolic extract of *Manihot esculenta* leaves.

In our results correlation was observed between total phenolic and ascorbic acid content. An increase in reducing power was observed in methanol extract followed by acetone extracts of cassava leaves shown in Table 1. Ascorbate has been found chloroplast, vacuole and extracellular compartments of plant cell and shown to function as a reductant for many free radicals. The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking free radical chain by donating a hydrogen atom (Suganyadevi *et al*., 2011). The β carotene was high in methanol extract than the acetone extract of Cassava leaves. This may be due to high phenolic content found in methanolic extracts of *Manihot esculenta* leaves.

The efficacy of current antimicrobial agents has been reduced due to the pathogens to commonly used antimicrobials. Therefore the searches for new drugs from existing antibiotics are being threatened by the emergence of multidrug - resistant pathogens. The antimicrobial property of 2 different extract of cassava leaves were analyzed against 5 bacterial strains using Spectinomycin (antibiotic) as control. The antimicrobial activity of the plant extract against microorganism examined was assessed by the presence or absence of inhibition zones after 24 hours. The maximum activity was observed in methanolic extract of Cassava leaf (100 %) against *Staphylococcus aureus* (16 mm) is a gram positive bacteria. The least activity was observed in acetone extract against *Pseudomonas aeruginosa* (2.5 mm) is a gram negative bacteria (Table 2). Isnatin *et al* ., (2013) reported that the ethanol extract of the plant leaves demonstrated better antimicrobial activity compared to water extract which produced inhibition against three of the tested bacteria (*B. cereus, S. dysenteriae and Salmonella typhi*).

The present study confirms the presence of valuable antioxidants present in the plants further thorough studies may bring out the real potential of these widely used medicinal plants in preparation of antibiotic, antioxidant and antibacterial drugs.

Table 1: Total Phenolic and Antioxidant Analysis of Cassava leaves

Assays	Methanol extract	Acetone extract
Total Phenol (mg/g)	105±0.02	98±0.03
Ascorbic acid(mg/g)	0.58	0.48
Reducing power (%)	96.42	81.26
β carotene (%)	67	42

Table 2: Antimicrobial activity of Cassava leaves

Organisms used	Methanol extract (mm)	Acetone extract (mm)
S. aureus		
Control	9	9
100%	14	4
75%	11	3
50%	8	3
Solvent	1	0
E. coli		
Control	11	11
100%	2	4
50%	1	3
25%	2	3
Solvent	0	0
K. pnuemoniae		
control	6	6
100%	2	2
50%	2	2
25%	2	1
Solvent	0	0
P. aeruginosa		
Control	8	8
100%	1	1
50%	1	0
25%	1	0
Solvent	0	0
Serratia		
Control	4	4
100%	3	3
50%	2	4
25%	1	2
Solvent	0	2

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