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Research Article

Biochemical Quantification and antibacterial properties of *Corallocarpus epigaeus*

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Abstract

In this study was under taken to quantify the nutritional contents and antibacterial properties of tuber in *Corallocarpus epigaeus* was found at different levels for the possible utilizations. To determine the total crude fiber (3.34±0.01), protein (2.57±0.20), ash (2.2±0.15), carbohydrate (91.92±0.50), fat (0.59±0.02), moisture (85.63±1.63), lipase (0.08µg/ml) and amylase (0.07µg/ml) were obtained various concentration respectively. Higher the carbohydrates content in the tuber (91.92±0.50) give a subsequent increase in the energy value (378.13±0.03 Kcal/100g) for the tuber. The antibacterial activity of the methanol, chloroform, ethyl acetate, aqueous and petroleum ether extracts were tested against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The ethyl acetate extract showed the best antibacterial properties by inhibiting the growth of tested organism followed by petroleum ether. There is no activity was showed against *Staphylococcus aureus* in methanol and chloroform extract. Whereas, chloroform, ethyl acetate and aqueous extract against *Pseudomonas aeruginosa*, which was compared with the ampicillin as a control. Therefore, these plants have been showed rich in some essential nutrient, especially carbohydrate and enzymes which are essential for human health care.

INTRODUCTION

Medicinal plant, traditionally has been largely indiscriminate without due regard to possible side effects. Diet has long been considered as the major source of human exposure to trace elements and consequently the levels in basic foodstuff, but medicinal uptakes are of greater inter from the toxicological and nutrient point of view (Dim *et al*., 2004). Plant foods can contribute significantly to human nutrition and health, because they contain almost all essential human nutrients. However, nutrient composition varies among different plant foods. Improvement of nutritional quality of our food supply, especially with respect to essential nutrient values (Arzani *et al*., 2007). Plants are potential sources of natural antioxidants (Lu et al., 1995). Macro and microelements influence biochemical processes in the human organism (Brouns and Vermeer, 2000). Study of elements with respect to indigenous medicinal plant reveals that major and trace elements have significant roles in combating a variety of human ailments and disease (Shirin et al., 2010) *C. epigaeus* (Hook. F.) belongs to Cucurbitaceae family. The *C. epigaeus* is tendril-bearing climbing herbs and the root is yellowish white; it has a bitter

taste and is credited with alterative and laxative properties, and is used in syphilitic rheumatism, later stages of dysentery (Kirtikar and Basu, 1984). Most plant based agricultural wastes are a rich source of dietary fiber and phytochemical which may be further utilized as functional ingredients. However, one major concern of utilizing agricultural waste as a feed is the presence of excessive phytochemical that may exert anti-nutritional or anti-physiological effects in animals (D'Mello, 2000). The present research was conducted to determine the nutraceuticals and antibacterial properties of *C. epigaeus* tuber.

MATERIALS AND METHODS

Collection and preparation of plant material

The fresh *C. epigaeus* tubers were collected and dried at room temperature, grounded to powder and finally stored in air tight containers for further use. The plant was extracted with (90%) methanol, chloroform, ethyl acetate, petroleum ether and aqueous (100%). Then, the solvent was distilled under reduced pressure in a rotary vacuum evaporator until the extracts became dry. The percentage (%) of yield extract was determined and the crude extract was then transferred in a bottle by sterile spatula (Anup, 2006).

Chemical and reagents

Soluble Starch Solution (1%), Sodium Acetate Buffer (pH 6.0), $CaCl_2$ (5mM), NaCl (0.04%), Dinitrosalycylic acid (DNSA), Phosphorus Buffer (0.1M), H_2SO_4 (1.25%), NaOH (1.25% and 40%), Boric acid (2%), Ammonia (NH_3), Hydrochloric Acid (0.1N and 2.5 N), Anthrone reagent, Muller Hinton Agar (MHA), Tween20 (2%) and methanol are the additional required solution.

Determination of moisture, ash, crude fiber, protein, fat and carbohydrate

The moisture content of the tuber was determined by drying of 2g of the tuber powder in oven at 105^0C for 6 hrs. The dried sample was removed and placed in desiccators to cool, and then weighed. The moisture content was then determined by difference and expressed as a percentage of the initial weight of the sample (AOAC, 1990). The total ash content was determined according to the method by Ceirwyn (1995). 2g was weighed from each of the samples dried, ignited and weighed Gouch porcelain crucible and then the dish with its contents placed in a Muffle furnace preheated to 600oC for 2 hrs until grayish white was obtained. The ash content was then calculated. Crude fiber was determined by acid bases digestion with 1.25 % H_2SO_4 (W/V) and 1.25 % NaOH (W/V) solutions. The contents was dried for 1hrs at 105^oC in a drying oven and weighed. This content was then ignited in a muffle furnace for 30 min at 60ºC and reweighed. The loss of weight was reported as percentage crude fiber (AOAC, 2010). Estimation of crude protein by kjeldahl method was determined by multiplying the value obtained from kjeldahl's nitrogen by a protein factor of 6.25 (AOAC, 1990). Crude fat was quantified by the method describe by Antia (2006), 2g sample was put in a paper thimble and plugged with cotton wool. The thimble was placed in a soxhlet extraction apparatus and extracted with petroleum ether (40-60^o C) and methanol mixed properly in the ratio 1:1, at low heat for 6 hrs in a continuous extraction manner. The extract was collected and dried at 100^o C, and weighed. The total carbohydrate content was measured by the method by Hedge and Hofreiter (1962). The available of carbohydrate was calculated by difference; total sum of crude fat, crude fiber, crude protein and ash deducted from 100% dry substance.

The energy values were quantified as the following formula:

Energy value (Kcal/100g) = (Crude fat x 9) + (Crude protein x 2) + (Carbohydrate x 4) (Asibey-Berko and Taiye, 1999)

(1)

Estimation of crude enzyme

Plant material were taken 25g and washed thoroughly under running tap water and 2% Tween20 for 20 min. It was then rinsed with distilled water and was homogenized in sodium acetate buffer. The homogenate was filtered through muslin cloth. The filtrate was collected and clarified further by centrifugation at 5000rpm for 20min at 4^0C. The supernatant was collected and stored at 4^0C for further enzyme assays. Total amylase activity was determined by dinitrosalycylic acid (DNSA) assay of Bernfeld (1955). Lipase activity was measured by emulsified free system method by Sadasivam and Manikam (1996). The flask containing 2ml of 0.1M of phosphorus buffer, 1 ml of olive oil and 1ml of crude enzyme extract was incubated at 40ºC for 30min. The reaction was stopped by the addition of 5ml of ethanol and it was titrated against 0.1N NaOH using phenolphthalein as indicator. The appearance of pale pink color was the end point. Lipase activity was calculated using the following formula:

Lipase activity = µg/ml/min= volume of alkali consumed X normality of NaOH / Time of incubation X volume of enzyme solution ……. (2)

Nutrient density

Contribution of RDA (%) = Concentration of the element / RDA X 100 …… (3)

RDA = Recommended Dietary Allowance (Pamela, 2005)

Test Organism

Plant extracts were used against following test organisms; *Pseudomonas aeruginosa* (MTCC-2295) and *Staphylococcus aureus* (MTCC-7443). All the stock cultures were obtained from Microbiology lab, University of Madras, Chennai, India.

Culture media and inoculums preparation

Muller Hinton Agar (MHA) was dissolved in distilled water and the volume was made up to 300 ml. The media was autoclaved for 15 min at 15 psi pressure at 121ºC. The plates were prepared and pouring 20 ml of media in petriplates and the plates were allowed to solidify. 0.1% of inoculums were swabbed uniformly and the inoculum was allowed to dry.

Antibacterial activity

The zone of inhibitory activity was done by agar well diffusion method (Arumugam *et al*, 2011) to determine the inhibitory activity of the tested extracts. Agar well of 6mm diameter were made in the plates. The different concentrations (2.5, 5, 7.5 and 10mg/ml) of each extracts were added to each well into Muller Hinton Agar (MHA) plates already seeded with the standardized inoculums of the test bacterial cells. All test plates were incubated at 37°C for 24h. The respective solvent control for tuber extracts was also maintained and the diameter of zone of inhibitions were recorded in mm and compared with standard values. Triplicates were done and the experiment was repeated thrice.

Statistical analysis

The experimental results were expressed as mean ± standard deviation (SD). Each value is expressed as mean of triplicate experiments.

RESULTS AND DISCUSSION

The nutrient values in *C. epigaeus* showed summarized in Table 1. An examination of data Table 1 shows that the nutrient value contents in analyzed *C. epigaeus* tuber are an extensive range. Highest total carbohydrate (91.92±0.50 was found, followed by Moisture content (85.63±1.63), whereas the Crude fiber contains (3.34±0.01), the amount of protein was found (2.57±0.20) and least amount of ash (2.2±0.15) respectively. Fat was found much lower (0.59±0.02) in *C. epigaeus* tuber. The result was promising to make a note that *C. epigaeus* tuber are very rich amount in carbohydrate followed by moisture, crude fiber and protein respectively (Figure 1).

Table 1 shows the data on the nutritional value of *C. epigaeus* tuber. The moisture content of the tuber was found (85.63±1.63). This value is higher than that reported for *Amaranthus cruentus (*23.6±4.10), *Celusia argenta* (15.60±1.0) and *Corchorus olitorius* (30.90±1.30) (Onwordi *et al*, 2009). The moisture content depends on climatic factor and the process used for drying. Several workers reported wide variation in moisture content in tuber starches (Moorthy, 2002).

The ash content of the *C. epigaeus* tuber was obtained to be 2.2±0.15 which is lower than the value of *Amaranthus cruentus* (19.3±5.7), reported by Fasuyi (2006). Ash in plant contributes the residue remaining after all the moisture was removed as well as the macrobiotic material (protein, fat, vitamins, carbohydrates, organic acid etc.) have been incinerated at a temperature. Ash substance is usually taken to be determining of the mineral content of the original food (Onwuka, 2005).

The crude fiber content of the tuber (rhizome) powder shown as Figure 1 was found 3.34± 0.01. The fiber content of *Celusia argenta* (11.70±0.80) and *Corchorus olitorius* (6.70±1.40) were reported (Onwordi et al, 2009). Crude fiber in plant is an indication of the intensity of non-digestible lignin and carbohydrate. The lower level is considered appropriate, since it aids absorption of glucose and fat. While crude fiber enhances digestibility, its presence in high level can cause intestinal irritation, lower digestibility and decreased nutrient usage (Oladiji *et al*., 2005). Similarly the present study showed minimum amount of fiber (Table1), so it can be used as digestibility agent in food product. The *C. epigaeus* tuber fat content of 0.59±0.02 was lower than the *Ocimum gratissimum* (3.00±0.15) (Figure 1). The result showed that the tuber of *C. epigaeus* have poor sources of fat, which are low fat containing foods, thus the great advantage health care in avoiding obesity (Lintas, 1992).

Table1: **Estimation of nutrient values and enzymes in *C. epigaeus* tuber**

Plant Sample	Parameter	Nutritive value (%)
Corallocarpus epigaeus	Moisture*	85.63±1.63
	Fat	0.59±0.02
	Total carbohydrate	91.92±0.50
	Protein	2.57±0.20
	Total ash	2.2±0.15
	Crude fiber	3.34±0.01
	Calorific (Energy) value (Kcal/100g)	378.13± 0.03
	Enzymes	**(µg/ml)**
	Lipase	0.08±0.00
	Amylase	0.07±0.01

Each value is expressed as mean ± standard deviation (SD) of triplicate determination.
* Value expressed as % wet weight

Table 2: **Antibacterial assay for zone of Inhibitory activity on *C. epigaeus* tuber extracts**

Extracts	*Staphylococcus aureus* Zone of inhibition (mm)					*Pseudomonas aeruginosa* Zone of inhibition (mm)				
	2.5 mg	5 mg	7.5 mg	10mg	Ab*	2.5 mg	5 mg	7.5 mg	10mg	Ab*
Methanol	-	-	-	-	20.03±.01	12.08±0.1	14.03±0.1	16.98±0.1	19.06±0.0	19.02±0.1
Chloroform	-	-	-	-	19.02±0.1	-	-	-	-	18.01±0.1
Ethylacetate	17.01±0.2	19.1±0.3	21.03±0.1	22.96±0.1	18.01±0.1	-	-	-	-	18.01±0.1
Aqueous	-	-	17.0±0.1	19.01±0.1	20.01±.01	-	-	-	-	19.03±0.1
Petroleum ether	-	-	-	15.01±0.1	17.0±0.1	15.05±0.1	17.01±0.1	19.11±0.0	22.0±0.1	18.05±0.1

Each value is expressed as mean ± standard deviation (SD) of triplicate determination
- No activity; *Ab- Ampicillin

The lipid provides very good sources of energy and transport of fat soluble vitamins, insulates and protects internal tissues and contributes to important cell processes (Jones *et al*., 1985, Pamela *et al*., 2005), it is good to add fat to most of our diets.

The crude protein of *C. epigaeus* tuber was present (2.57±0.20) dry weight of nutritive value. Proteins act as enzymes, hormones, and antibodies and it is responsible for the formation of bones, teeth, hair and the outer layer of skin and they help maintain the structure of blood vessels and other tissues (Protein, 2010).The recommended dietary allowance for protein is 56g for individual weighing 70kg and 46g for adult weighing 50kg; children may consume 2kg/day (Jones *et al*., 1991). The plants have reasonable resource of protein and proteins from plant sources have low, while compare to animal protein may result in sufficient nutritional value (Pamela *et al*., 2005).

Carbohydrates are the human body key source of energy. The study revealed that the carbohydrate contents of *C. epigaeus* tuber to be 91.92±0.50. The high levels of carbohydrates were presence in this plant is given the (Table 1 and Figure 1). The plants contains moderate source of carbohydrate, while compared to Recommended Dietary Allowance (RDA) of 130g (Pamela *et al*., 2005). These results revealed that, the *C. epigaeus* tuber contain considerable amount of nutrient and it should be included in diets to supplement our body needs.

The *C. epigaeus* tuber showed great promise as a dietary resource of these human essential nutritive values especially to village people and who's depending on herbal medicine resource. In this study revealed that, this medicinal plant (tuber) possesses the significant amount of nutrient contents were found at different level.

The concentration levels of enzymes were found in *C. epigaeus* tuber is shown in (Table 1). The levels of enzymes present in tuber was ranged for lipase (0.08µg/ml), whereas amylase (0.07µg/ml). The enzymes concentrations in *C. epigaeus* are below measurable limit respectively. Low level of lipase (0.08µg/ml) and amylase (0.07µg/ml) was obtained in *C. epigaeus* tuber (Table 1). Taking plant enzymes with meals allows digestion to start in the stomach. Plant based enzyme preparation derived from plant sources such as lipase and amylase (Mario Roxas, 2008); it is simply support good digestive function.

The result obtained from our present study indicates that, the *C. epigaeus* tuber has the capability of analysis of nutrient contents as well as antibacterial activity. The antibacterial activity of tested various extract of *C. epigaeus* revealed significant effect in bacterial growth in terms of zone of inhibitory activity. All the tuber extracts observed dose depended activity, while increasing in the concentration of each extract, the zone of inhibition is also increased (Table 2). In the present investigation revealed that, the maximum growth of inhibitions (22.96 ±0.1mm) were observed in ethyl acetate extract of tuber at 10mg/ml against *S. aureus*, and *P. aeruginosa* (22±0.1mm) were observed in petroleum ether extract and which was followed by methanol extract (19.06±0.0mm) in 10mg/ml concentration respectively (Figure 2). Whereas the minimum growth of inhibition (12.08±0.1mm) was observed in methanol extract against *P. aeruginosa* and followed by petroleum ether extract (15.01±0.1mm) was measured against *S. aureus* respectively (Table 2). Notably, there is no significant activity was observed against *S. aureus* in the extract of methanol and chloroform. Similarly, chloroform, ethyl acetate and aqueous extract against *P. aeruginosa*, which was compared with the control as ampicillin. Jagtap *et al*. (2009) reported that, the aqueous extract of *C. asiatica* did not showed any antibacterial effects at lower concentrations, similarly in aqueous extract of *C. epigaeus*. But it was effective at the concentrations above 7.5 mg/ml against *S. aureus* (Table 2). The tuber extract of *C. epigaeus* showed inhibitory effect it may be due to the reason that, the tubers have constant contact with soil, though; they may be infected with soil pathogen. As a result, they produce many antimicrobial substances in response to the infection (Kelamanson *et al.*, 2000). The broad spectrum antibacterial activities of the tuber extracts, possibly due to the presence of phyto-molecules in the tuber. Thomas *et al*., (1989) reported that plant derived natural products offer clues to synthesize new structural types of antibacterial chemicals that are relatively safe to human being and it can facilitate to meet their expensive and supply of synthetic chemicals. This was broadly proved in many studies, which include enzymes, carbohydrates, alkaloids, glycoside, tannins, flavonoids, vitamins and protease (Dubey and Jagannadham, 2003).

The tuber extracts of *C. epigaeus* evidently shown the antibacterial properties and it's an evident that the findings of the present investigation can be give a basic idea for developing a new drug. This bacterial activity might be the presence of bioactive metabolites in this tuber. Thus, the study concluded that the value of the plant, which would be of great interest to the development of new drugs. It will also help to isolate bioactive compound that would be control the infectious disease causing pathogen in present and future.

Conclusion

Tuber (rhizomes) of *C. epigaeus* was analyzed using standard methods of food analysis. The result showed diverse level of total crude fiber, total, carbohydrate, moisture and ash content and enzymes. Carbohydrate content showed higher in the rhizome which gives a greater calorific value for the rhizome of this plant. Therefore, they can be utilized as pre-biotic compound and *C. epigaeus* rhizome may be utilized as antioxidants in nutraceutical sector in food industries.

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