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Evaluation of Nutritional, Antifungal Properties and Activities of Hydrolytic and Oxidative Enzymes of *Coccinia cordifolia* Leaves

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Authors' contributions

This work was carried out in collaboration among all authors. Author MSR designed the study and wrote the protocol. Author AN managed the animals, collected all data, performed the statistical analysis, and wrote the first draft of the manuscript. Author MA did the literature search and also wrote part of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Objective: This study demonstrated the significant changes of nutritional compositions, hydrolytic and oxidative enzymes of *C. cordifolia* leaves at different maturity stages.
Methods: Biochemical screening revealed the presence of reducing sugar, non-reducing sugar, starch, crude fibers and pectin in moderate concentration. Thiamin and riboflavin were estimated by Anonymous and β-carotene was estimated by Jensen. Cupper and magnesium content were determined by Atomic Absorption Spectroscopic method. Phosphorus was determined by colorimetric means. The protease and amylase activity were measured by Kunitz and Jayaraman respectively. Invertase activity was assayed by Mahadevan and Sridhar.
Results: Reducing sugar and pectin contents increased rapidly while starch content decreased with maturation. Non-reducing sugar and crude fiber content increased up to the mature stage and

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decreased in ripen stage. The activity of amylase and invertase increased up to mature and thereafter decreased in ripen stage. Polyphenol oxidase and peroxidase activity were high in immature stage but decreased in matured stage and thereafter increased in ripen stage while the activity of protease and lipase increased all the maturity stage.

Conclusion: *C. cordifolia* plant leaves demonstrated the variety amount of reducing sugar, non-reducing sugar, starch, pectin, crude fibers, β-Carotene, vitamin B_1, vitamin B_2 and several enzymes in the three different maturity stages.

Keywords: C. cordifolia leaves; nutrients; enzymes; vitamin; mineral; maturation.

1. INTRODUCTION

The World Health Organization (WHO) estimates that about 80% of the populations living in the developing countries rely almost on traditional medicine for their primary health care needs. Plants have played a significant role in maintaining human health and improving the quality of human life [1]. The Cucurbitaceae family is commonly known as gourd, melon and pumpkin family. The family of *Coccinia grandis* is Cucurbitaceous, comprises 960 species. The family is predominantly distributed around the tropics. Most of the plants in Cucurbitaceae family are annual vines [2]. The earlier authors demonstrated that the plant has hypolipidemic [3], antimutagenic [4], hypoglycemic [5] and anti-inflammatory [6] activities. This plant is also used in various skin diseases, bronchitis, small pox, ring worm, scabies [7] and ulcers [8]. Antimicrobial activities of *C. grandis* leaf and fruit extracts against several bacterial and fungal strains have also been reported [9,10]. *C. cordifolia* is a good source of calcium, protein and fiber [11,12]. It also contains beta carotene [13]. During maturation and senescence of fruits, proteolytic and hydrolytic enzymes play an important physiological role [14,15]. There are very limited research about the species has been done in board and aboard on the antifungal activity and physico-chemical compositions of different maturity stages of *C. cordifolia* leaves. Therefore, the present study designed to obtain information on the nutritional quality and the chemical compositions as well as the activities of some enzymes at three different maturity stages of *C. cordifolia* leaves.

2. MATERIALS AND METHODS

2.1 Plant Material

The leaves of *C. cordifolia* were collected at different maturity stages in December, 2014 from Rajshahi city in Bangladesh and authenticated by Botany Department, Rajshahi University. The leaves were initially dried under shade and grinded.

2.2 Preparation of Extract

The shade-dried leaves were coarsely powdered and extracted with 95% methanol and ethanol by a Soxhlet apparatus at 45ºC. The solvents were completely removed by rotary evaporator and obtained greenish gummy exudates. These crude extracts were used for further investigation.

Fig. 1. *Coccinia cordifolia* leaves at different maturity stages

2.3 Qualitative Analysis

Qualitative phytochemical tests were conducted for the identification of alkaloids, flavonoids, terpenoids, saponins, polyprenols and cardenolides in the *C. grandis* extract [16-19].

2.4 Nutritional Analysis

Reducing sugar content of the *C. cordifolia* leaves was determined by dinitrosalicylic acid method [20]. The starch content of the *C. cordifolia* leaves was determined by the Anthrone method [21]. The increases in reducing sugar were due to enzymatic conversion of starch to reducing sugar and also conversion of some non-reducing sugar [22]. Crude fiber was determined by the following method described by Neubert [23].

The vitamins, such as thiamin and riboflavin were estimated following the procedure as described by Anonymous [24] while β-carotene were estimated following the method described by Jensen [25]. The minerals such as, sodium, potassium, cupper and magnesium content were determined by Atomic Absorption Spectroscopic method of Issac and Johnson (1975). Phosphorus was determined by colorimetric means [26].

2.5 Enzyme Assay

20 gm of *C. cordifolia* leaves from each maturation stage were taken in a motor and pestle and then homogenized well with cold 0.1 M phosphate buffer of respective pH (amylase, pH 6.7, protease, pH 7.0, invertase, pH 7.0), while for the measurement of lipase 50 mM acetate buffer, pH 5.6 was used. After centrifugation at 8000g, 4ºC for 10 min. the clear supernatant was used as crude enzyme extract. The protease activity was measured by the method of Kunitz [27] while the activity of amylase was determined as per Jayaraman [28]. Invertase activity was assayed following the modified method as described in methods in physiological Plant Pathology [29].

2.6 Antifungal Activity

Methanol, Ethanol and Water extracts were prepared to evaluate the zone of inhibition against individually test microorganisms. Extract inseminated disc was previously prepared at the concentration (6 mm in diameter), 1500 µg/ml for fungi were placed on sensitivity plates with controls. Griseofulvin (2000 µg/ml) was used as positive control for fungi. Then, the sensitivity plates were incubated at 30ºC for 3 days for fungal spores [30]. By the measurement of the clear zone surrounding the disc on agar surfaces, the antifungal activity of the extract was recorded.

3. RESULTS AND DISCUSSION

Phytochemical screening of the extract of *C. cordifolia* revealed the presence of various bioactive components of which alkaloid, cardenolides, flavonoids, saponins and polyprenols were the most prominent (Table 1). These compounds have been reported to possess antibacterial activity [31-33].

Reducing sugar, non-reducing sugar, starch, pectin and crude fiber contents of *C. cordifolia* leaves in three different stages were shown in Table 2. The result indicated that the reducing sugar and pectin content of *C. cordifolia* leaves increased significantly with the change of maturity and non- reducing sugar increased in mature stage and then decreased in ripen stage. These results were similar to the finding of Abdullah et al. [34] who reported that total sugar content of banana increased with the change of maturity.

Starch content was high in immature stage but decreased in matured stage and thereafter increased in ripen stage. The reduction of starch with the change of maturity might be due to the hydrolysis of starch, which shows good correlation with the increase in the contents of total soluble sugar [35]. Crude fiber was increased in mature stage and then decreased in ripen stage.

The vitamins and minerals content were shown in Table 3. It was found that *C. cordifolia* plant leaves are good sources of vitamins. Vitamin B1 (0.20-0.42 mg%), Vitamin B2 (0.13 -0.19 mg%), and ß-Carotene (605-1125 µg%). The major minerals analyzed in *C. cordifolia* leaves are potassium (3.8-7.9%), calcium (48.84-56.35 mg%), sodium (6.1-6.6%), copper (16.00-24.00 mg%) and magnesium (29.00-37 mg%). All minerals content significantly increased up to the mature stage and then decreased in ripen stage.

Activities of some hydrolytic and oxidative enzymes in *C. cordifolia* leaves were shown in Table 4. The results demonstrated the significant activities of amylase, protease invertase and

lipase in different maturity stages of *C. cordifolia* leaves were found between 7.0 to 12.2 mg%, 0.55 to 3.35 mg%, 0.29 to 1.77 mg% and 2.0 to 7.1 mg% respectively. Activities of amylase and invertase increase significantly up to mature stage and thereafter decreased significantly, while the activity of protease and lipase increased with the advancement of maturity. Among the hydrolytic enzymes, lipase showed the highest activity at ripens stages. Polyphenol oxidase and peroxidase activity increased greatly in immature stage and then decreased dramatically in mature stage and thereafter increased in ripen stage.

Table 1. Phytochemical investigation of ethanol extract of *C. cordifolia* leaves

Test	Alkaloids	Cardenolides	Flavonoids	Terpenoids	Saponins	Polyprenols
Observation	+	++	+++	+	+++	+

'+++'= indicates presence in high concentration; '++'= indicates presence in moderate concentration; '+'= indicates presence in trace concentration

Table 2. Reducing sugar, Non-reducing sugar, starch, pectin and crude fibers contents of *C. cordifolia* leaves at different maturity stages

Parameters	Stage of maturation		
	Immature	Mature	Ripen
Reducing sugar (gm%)	0.83	0.98	1.65
Non-reducing sugar (gm%)	0.38	0.62	0.55
Starch (gm%)	5.20	3.01	1.48
Pectin (mg%)	0.72	0.77	1.19
Crude fibers (gm%)	2.24	2.94	2.04

Table 3. Vitamins and minerals content of *C. cordifolia* leaves at different maturity stages

Parameters	Stages of maturation		
	Immature	Mature	Ripen
Vitamin B1 (mg%)	0.20±0.14	0.25±0.34	0.42±0.22
Vitamin B2 (mg%)	0.13±0.04	0.15±0.07	0.19±0.02
ß-Carotene (µg%)	605±0.29	876±0.11	1125±0.18
Potassium (%)	3.8±0.02	7.9±0.06	3.7±0.07
Sodium (%)	6.1±0.12	5.80±0.05	6.6±0.08
Cupper (mg%)	16.00±0.58	24.0±0.36	22.00±0.28
Magnesium (mg%)	29.20±0.05	37.45±0.08	29.00±0.09

Values are mean ± S.D. of triplicate analyses

Table 4. Activities of amylase, protease, invertase, lipase, and peroxidase, polyphenol oxidase enzymes of *C. cordifolia* leaves at different maturity stages

Name of Enzymes	Stages of maturation		
	Immature	Mature	Ripen
Amylase (unit gm-1 leaves).	7.0±0.01	12.2±0.07	8.1±0.02
Protease (unit gm-1 leaves).	0.55±0.03	1.05±0.08	3.35±0.02
Invertase (unit gm-1 leaves).	0.29±0.01	1.77±0.03	0.21±0.01
Lipase (unit gm-1 leaves)	2.0±0.06	6.21±0.08b	7.10±0.02
Peroxidase (unit min-1gm-1 leaves)	16.10±0.03	0.9±0.15	11.30±0.04
Polyphenol oxidase (unit min-1gm-1 leaves)	12.80±0.09	0.6.±0.02	0.8±0.03

Values are mean ± S.D. of triplicate analyses

Table 5. Antifungal activity of *C. cordifolia* plant leaves extracts

Fungi	Methanol extract (1500 µg/ml)	Water extract (1500 µg/ml)	Ethanol extract (1500 µg/ml)	(Griseofulvin 2000 µg/ml)
Candida albicans	12.2±0.2	6.2±0.2	8.0±0.5	17.6±0.4
Aspergillusniger	7.8±0.1	4.9±0.1	6.3±0.2	14.4±0.2
Penicillium notatum	5.8±0.5	4.8±0.3	6.7±0.1	11.5±0.3

Values are mean ± S.D. of triplicate analyses

The antifungal activity of *C. cordifolia* plant leaves extracts was shown in Table 5. The methanol, ethanol and water extracts showed significant antifungal activity against three testes fungi compared with Griseofulvin standard. The methanol extract showed high activity against *Candida albicans, Aspergillus niger,* and *Penicillium notatum* and the zone of inhibition were 12.2, 7.8 and 5.8, respectively. The ethanol extract showed highest antifungal activity against the same fungi as *Candida albicans, Aspergillus niger* and *Penicillium notatum* and the zone of inhibition were 8, 6.3 and 6.7, respectively.Water extract showed lowest activity and the zone of inhibition were 6.2, 4.9 and 4.8, respectively. The phytochemicals present in the plant leaves were responsible for the antifungal activity.

4. CONCLUSION

Based on the results obtained, the ripen *C. cordifolia* plant leaves might be considered as nutritionally rich source since it contained the highest amount of reducing, non-reducing sugar, β-Carotene, vitamin B1, vitamin B2 and whereas mature and immature *C. cordifolia* leaves are rich sources of minerals and starch respectively. Enzymes present in the plant leaves are necessary for digesting food, for stimulating the brain, for providing cellular energy, and for repairing all tissues, organs, and cells. *C. cordifolia* plant leaves extracts demonstrated significant inhibition effects against some tested fungi. Therefore, the *C. cordifolia* plant leaves might be used as potential nutritional source as well as chemotherapeutic agent. This is an ongoing study and further work is being carried to investigate its biological activities.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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