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Phytochemical Screening, Determination of Total Phenolic and Flavonoid Content and Antioxidant Activity of Methanolic Extract of *Chassalia curviflora* Leaves

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Abstract
The study was carried out to assess the phytochemicals and antioxidant activity of the methanolic extract of *Chassalia curviflora* leaf part. Phytochemical tests were done by various chemical methods to determine the presence of chemical compounds. Total phenolic content, total flavonoid content, and DPPH scavenging assay method were used to evaluate antioxidant potentials of *Chassalia curviflora* methanolic leaf extract. In phytochemical screening tannins, alkaloids, flavonoids, saponins, steroids, glycosides were found. The phenolic content of methanol extract of *C. curviflora* leaf was 265 mg of GAE/gm of dried extract. The Flavonoid content was 300 mg of GAE/gm of dried extract. In the DPPH method, *C. curviflora* leaf showed better antioxidant potentiality with the IC_{50} value of 11.36µg/ml compared to standard BHT 9.24 µg/ml. The results demonstrated that the methanolic extract of *Chassalia curviflora* leaf had the potential phytomedicinal value for its significant antioxidant activity.

Keywords: Antioxidant Activity, *Chassalia curviflora*, Methanolic Extract

I. Introduction

Medicinal plants have been used in healthcare since ancient times. The global industry for medical plant products is worth more than $100 billion each year (A. Sofowora *et al*., 2013). An antioxidant is a molecule that inhibits the oxidation of other molecules and protects the cell against the damaging effects of reactive oxygen species, thus they act as "free radical scavengers". Oxidative damage has a role in a variety of health conditions, including heart disease, muscle degeneration, diabetes, and cancer. (L. A. Pham-Huy *et al.*, 2008). The antioxidants whether natural or synthetic, are crucial health-protecting factors. Plants with dark color fruits are the major source of antioxidants (K. Krishnamoorthy *et al.*, 2013).

Chassalia curviflora also known as Psychotria *curviflora* belonging to the family Rubiaceae. Crushed leaves are used in snake and insect bites by the Chakma tribal people of Bangladesh. The people of the Kani tribes of Agasthiyamalai, Kerala, use it in the treatment of jaundice. Both the root and leaf have anti-inflammatory and analgesic properties (K. Krishnamoorthy *et al.*, 2013, L. A. Pham-Huy *et al.*, 2008). The root is also reported to be used in rheumatism, pneumonia, malaria, cough, and phlegm (G. R. Gopal *et al.*, 2016). Although there is little phytochemical research on secondary metabolites in *C. curviflora*, some *Chassalia* species are a source of macrocyclic peptides, also known as cyclotids. (K. R. Gustafson *et al.*, 1994). *Chassalia* genus was reported for its anti-hypertensive (W. N. H. W. Kadir and N. A. M. Omar, 2014), antibacterial (D. A. R. Rajendran *et al.*, 2011), antimicrobial, insecticidal and cytotoxic activities (P. A. Onocha and S. M. Ali, 2010).

In another study, Thenmozhi and co-workers detected the presence of phenols, tannins, and flavonoids in the fruits of *C.* curviflora (K. Krishnamoorthy *et al.*, 2013). GC/MS analysis of methanolic extract of *C.* curviflora revealed the presence of 69 phytoconstituents (W. N. H. W. Kadir and N. A. M. Omar, 2014). It is supported by numerous pieces of evidence that the medicinal values of a natural extract are always related to its phytochemical constituent.

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To be proved efficacious as an antioxidant, analgesic, anti-hypertensive, anti-inflammatory, and antimicrobial agent, higher animal model studies are required.

II. Aim and Objective

To characterize the chemical constituents and evaluate the antioxidant features of methanolic extract of *C. curviflora* leaves.

III. Materials and Methods

A. Plant material collection and identification

The leaf sample of *C. curviflora* was collected during October 2017 from Chandpur, Bangladesh.

B. Drying and Grinding

After separating the undesirable plant parts, the sample was washed properly and then sun-dried for several days. Using a high-capacity grinding machine, the dried materials were ground into a coarse powder.

C. Extraction

250 gm of the powdered sample was soaked in methanol (1.5 liters) in a clean amber glass bottle for 10 days. Occasional shaking and stirring were done. After that, the entire mixture was filtered using cotton and Whatman No. 1 filter paper. The obtained filtrate was concentrated in a rotary evaporator at 50°C temperature to get the crude extract. The weight of the air-dried, concentrated crude methanolic extract of *C. curviflora* (MECC) was 14gm.

D. Phytochemical Screening

Phytochemical screening of crude MECC extract was done according to the description of standard protocol to detect the presence of chemical constituents (G. R. Gopal *et al.*, 2016, S. T. Shanmugam *et al.*, 2010). Alkaloids were detected with Dragendroff's reagent, Mayer's reagent, and using picric acid, flavonoids detected with Mg and HCl, tannins detected with ferric chloride, the capacity to form stable foam indicated the presence of saponins.

E. Determination of Total Phenolics

The total phenolic content of the extract was measured using the Folin-Ciocalteu reagent as an oxidizing agent and Gallic acid as a standard, as described in the method (V. L. Singleton and J. A. Rossi, 1965).

F. Determination of Total Flavonoid

Total Flavonol in the leaf extract was estimated using the method of A. Kumaran and R. J. Karunakaran, 2007. The total content of Flavonoid was expressed in terms of Gallic acid equivalent, GAE. (Standard curve equation: y= (c x V)/m,) mg of GAE/g of dry extract (A. Kumaran and R. J. Karunakaran, 2007, P. Prieto *et al.*, 1999).

G. DPPH Scavenging Assay

DPPH scavenging activity of *C. curviflora* extract at different concentrations was determined by the method as described by (M. S. Ahammed and M. S. Islam *et al.*, 2018) with a slight modification.

The capacity of 1,1-diphenyl-2-picryl-hydrazyl (DPPH), a persistent free radical, to decolorize the presence of antioxidants is used in the DPPH antioxidant assay. The degree of decolorization can be determined using the change in absorbance and the percent of scavenging activity. (T. B. Nguelefack *et al.*, 2011). If the MECC sample possesses any scavenging property, the activity will increase with the increase of sample concentration.

IV. Results

A. Phytochemical Analysis

The presence of tannins, flavonoids, terpenoids, steroids, glycosides, alkaloids, saponins has been shown qualitatively in Table 1.

Table 1: Phytochemical test observations of crude MECC

Sl. No.	Name of Phytochemical	Observations
1	Tannins	+++
2	Flavonoids	+
3	Terpenoids	+++
4	Steroids	+
5	Glycosides	+
6	Alkaloids	++
7	Saponins	+++

Here, + = Present in low amount
++ = Present in moderate amount
+++ = Present in high amount

B. Result of Total Phenolic Content

The total phenolic content of the MECC was determined. Ascorbic acid was used as a reference standard in this analysis.

Preparation of Standard Curve

A plot was constructed using the absorbance of different concentrations; a linear relationship was established, as illustrated in Fig. 1. This curve is known as the standard curve.

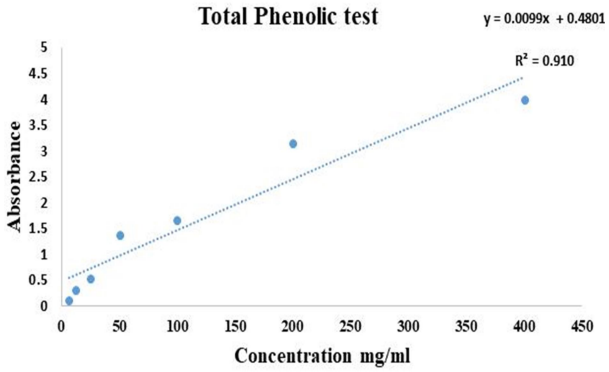

Figure 1: Gallic acid standard curve for total phenolic content measurement

Total Phenolic Content Present in Extract

Table 2: Total phenolic content of crude MECC

Concentration (mg/ml)	Absorbance	Amount of phenolic content (GAE/gm of dried extract)
2	1.005	265

265 mg of GAE/gm of dried extract of phenolic content was discovered at crude MECC concentration of 1 mg/ml was found from the standard curve.

C. Result of Total Flavonoid Content

To calculate flavonoid contents in the extract, the reference standard was gallic acid. Gallic acid absorbance at various concentrations was measured after treatment with the Folin-Ciocalteu reagent. The total flavonoid content of crude MECC was determined.

Preparation of Standard Curve for Total Flavonoid Content

A linear relationship was found through plotting the absorbance of different concentrations of Gallic acid solution which was shown in Fig. 2. The obtained linear curve was thought to be a standard one.

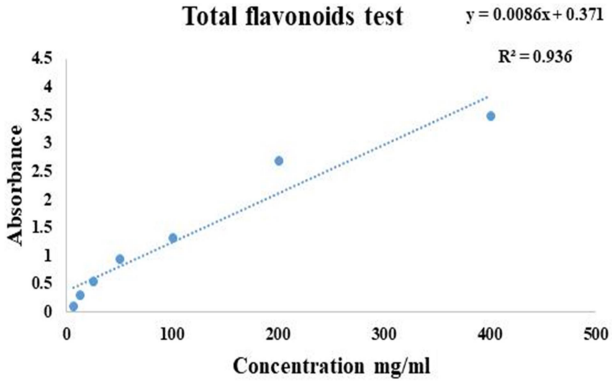

Figure 2: Gallic acid standard curve for the determination of flavonoid contents

Total Flavonoid Content Present in Extract

Table 3: Total flavonoid content of crude MECC

Concentration (mg/ml)	Absorbance	Amount of flavonoid content (mg of GAE/g of dried extract)
2	0.887	300

For 1 mg/ml concentration of crude MECC, 300 mg of GAE/gm of dried extract of flavonoid content was found. So the obtained result indicated that the extract contains compounds having antioxidant properties.

D. DPPH (1,1-Diphenyl-2-Picrylhydrazyl) Radical Scavenging Assay

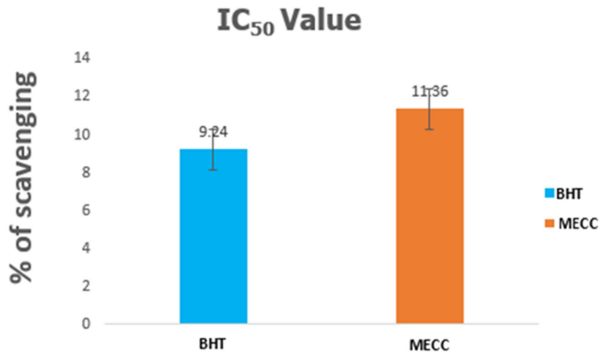

Figure 3: IC_{50} (µg/ml) values of BHT (Standard) and the MECC for DPPH radical scavenging activity

According to DPPH radical scavenging method, the antioxidant capacity of the sample extract is better than the standard BHT.

Table 4: DPPH radical scavenging activity of BHT (Standard) and the crude MECC extracts at different concentrations

Sample	Conc. (µg/ml)	% of Scavenging			% of Scavenging Mean ± STD	IC_{50} (µg/ml)
		a	b	c		
BHT	15.626	84.46	84.49	84.53	84.49 ±0.035	9.24
	31.25	88.76	88.71	88.82	88.76 ±0.055	
	62.5	92.45	92.54	92.41	92.46 ±0.066	
	125	93.65	93.76	93.62	93.67 ± 0.073	
	250	95.44	95.57	95.53	95.51 ±0.066	
	500	96.33	96.40	96.46	96.39 ±0.065	
MECC	15.626	68.67	68.76	68.73	68.72 ±0.045	11.36
	31.25	69.45	69.54	69.55	69.51 ±0.055	
	62.5	70.43	70.49	70.63	70.51 ±0.102	
	125	73.45	73.55	73.63	73.54 ±0.090	
	250	76.23	76.31	76.27	76.27 ±0.040	
	500	79.47	79.55	79.61	79.54 ±0.070	

V. Discussions and Conclusions

Free radicals have some serious deleterious effects on biological systems. Hence radical scavenging activity of antioxidants is so important to counter the damaging consequences of free radicals. Quantitative phytochemical analysis indicated that the plant contains a substantial amount of phenolics, flavonoids, and vitamin C, which may have a function in the neutralization of free radicals and quenching singlet and triplet oxygen and prevent the onset of diseases (J. Dai and R. J. Mumper, 2010). Here our experimental sample also showed promising antioxidant potentials. It also contained a considerable amount of phenolics and flavonoids content, which may be responsible for its better antioxidant properties. So, further investigations are needed to isolate and identify the active compounds responsible for antioxidant activity present in the sample extract.

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