

## Total antioxidant capacity and profiling of polyphenolic compounds in jute leaves by HPLC-DAD

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### Abstract

Jute leaves (*Corchorus* spp.) have been used as a medicinal plant for the treatment of various diseases. The study was investigated on the antioxidant activities and HPLC profiling of polyphenolic compounds in ethanol extract of *Corchorus olitorius* (*C. olitorius*) and *Corchorus capsularis* (*C. capsularis*) leaves. The total antioxidant capacity was evaluated by phosphomolybdenum method and Identification and quantification of polyphenolic compounds were performed using HPLC-DAD system. The results indicated that eight polyphenolic compounds were found in the *C. capsularis* leaves but *C. olitorius* leaves contain six polyphenolic compounds. In fact, major identified polyphenolic compounds of *C. capsularis* leaves were caffeic acid (CA), 55.93±0.13; *trans*-ferulic acid (FA), 58.02±0.18; rutin hydrate (RH), 32.16±0.08; ellagic acid (EA), 53.65±0.11 and quercetin hydrate (QU), 46.17±0.09 mg/100 g of dry extract respectively. Whereas in *C. olitorius* leaves which were rutin hydrate (RH), 152.17±0.51; ellagic acid (EA), 143.27±0.58 and quercetin hydrate (QU), 292.83±0.73 mg/100 g of dry extract respectively. The results showed that *C. capsularis* leaves contained high level of total antioxidant capacity (214.32±1.95 mg of ascorbic acid/g of dry extract) than that of *C. olitorius* (165.66±1.30 mg of ascorbic acid/g of dry extract) leaves. The overall data suggested that *C. olitorius* and *C. capsularis* leaves contain a significant amount of several polyphenolic compounds that could be used as a natural antioxidant for functional foods.

## 1. Introduction

Jute (*Corchorus* spp.) is a cash crop in Bangladesh being cultivated in 10% of agricultural land area (Islam, 2019). It is cultivated in many other countries like as India, Myanmar, Nepal, China, Taiwan, Thailand, Vietnam, Cambodia, Brazil, etc. *C. olitorius* jute namely is Tossa Jute and *C. capsularis* jute namely is White Jute (Islam, 2013).

Demand for medicinal plants is increasing in both developed and developing countries due to the growing recognition of natural products being equally effective, safe, non-narcotic, affordable and has no side effects.

One such medicinal plant part is jute leaves (Islam, 2013). Young shoots and leaves are eaten as vegetable and food ingredients and have long been used as medicinal folk remedies in East Asia and Africa. Health-flourishing effects of plant-derived secondary metabolites in human health, including antioxidative, anticarcinogenic, antibiotic, and pharmacological effects, are well documented (Lee *et al.*, 2015). *C. olitorius* leaves are used in the treatment of fever, tumors, pectoral pains, dysentery, aches, enteritis, cystitis, piles and dysuria (Adegoke and Adebayo-Tayo, 2009). *C. capsularis* leaves are also used in ayurvedic for ascites, piles, cystitis, dysuria, fever and gonorrhoea (Islam *et*

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al., 2013).

Leaves of *C. olitorius* have a large quantity of antioxidants compounds connected with various biological properties, which include diuretic, analgesic, antipyretic, antimicrobial activities, antitumor (Zakaria et al., 2006), phenolic antioxidative compounds (Azuma et al., 1999), hypoglycemic (Abo et al., 2008) and gastroprotective (Al Batran et al., 2013). On the other hand, *C. capsularis* leaves illustrated several pharmacological effects such as anticancer (Furumoto et al., 2002), antioxidant (Zakaria, 2007), anti-inflammatory, antinociceptive, antipyretic (Zakaria et al., 2009) and antimicrobial (Mondal et al., 2017).

Reactive oxygen species (ROS) could be characterized as signaling molecules and lead to oxidative-induced damage to cell membranes, protein denaturation, DNA mutations and lipid peroxidation (Beckers and Spoel, 2006), which are related to some chronic diseases, like cancer, inflammation, cardiovascular diseases and others (Pietta, 2000). Antioxidants may be defined as complex determined compounds that function as defensive shields against several diseases (Nath et al., 2013). Phenolic compounds are an important group of plant-based biologically active compounds that strengthen the organism and prevent disease (Sun et al., 2002; Gharras, 2009). Plant polyphenols are secondary metabolites characterized by one or more hydroxyl groups binding to one or more aromatic rings (Zhou et al., 2019). Phenolic compounds have a particularly strong antioxidant effect (Hider et al., 2001; Scalbert et al., 2005; Pandey and Rizvi, 2009). Numerous epidemiologic literature has verified an important correlation between the consumption of phenolic compound-rich food and a decreased risk for developing cardiovascular and other diseases (Weichselbaum et al., 2010; Spencer, 2010).

Hence, in this study, we attempted to investigate the total antioxidant capacity and HPLC profiling of bioactive polyphenolic compounds in 80% ethanol extract of two varieties of jute leaves growing in Bangladesh.

## 2. Materials and methods

### 2.1 Plant material

The two varieties of jute Leaves, *C. olitorius* (O-9897) and *C. capsularis* (CVL-1) were collected from Jute Seed Production and research centre, Bangladesh Jute Research Institute, Nashipur, dinajpur during June 2017. The leaves were properly washed to remove dirt and other impurities. After that, the leaves were dried under the shade. The dried leaves were powder by

pulverizes. The sample was then saved in an airtight container and storage in the refrigerator until extraction.

### 2.2 Extraction

The two varieties of shade dried leaves were extracted in an orbital shaker with 80% ethanol for 24 hrs at room temperature to obtain ethanol extract of jute leaves. The extract was initially filtered in a cotton plug to get rid of the plant debris and next through Whatman filter paper no 1. The solvent was removed using a rotary vacuum evaporator (R-215, Buchi, Switzerland) under reduced pressure. The concentrated filtrates were kept in the bottle at -20°C prior to further analysis.

### 2.3 Chemicals and reagents

All the standards were purchased from Sigma-Aldrich (St. Louis, MO, USA) and reagent was collected from Scharlau (Spain) and Merck (Germany).

### 2.4 Total antioxidant capacity

The total antioxidant capacity of the *C. olitorius* and *C. capsularis* leaves sample extract were evaluated by the phosphomolybdenum assay method (Prieto et al., 1999) which is based on the reduction of Mo (VI) to Mo (V) and the subsequent formation of a green phosphate-Mo (V) complex in acidic condition. 0.3 mL of each extract (1 mg mL<sup>-1</sup>) was allowed to mix with 3.0 mL of the reagent solution (0.6 M H<sub>2</sub>SO<sub>4</sub>, 28 mM Na<sub>3</sub>PO<sub>4</sub>, 4 mM ammonium molybdate). This reaction mixture was incubated at 95°C for 90 mins. After letting the solution cool back to room temperature, the absorbance was measured at 695 nm with a double beam UV/Visible spectrophotometer (Specord 205, Analytikjena, Germany) against a blank solution. The total antioxidant capacity was determined and expressed as mg ascorbic acid equivalents per gram of dry extract using the equation obtained from a standard ascorbic acid calibration curve.

### 2.5 Identification and quantification of polyphenolic compounds by HPLC

Identification and quantification of selected phenolic compounds in the 80% ethanol extract were determined by HPLC-DAD analysis as described by Hossain et al. (2016) with some modifications. It was carried out on a Dionex UltiMate 3000 system equipped with quaternary rapid separation pump (LPG-3400RS) and photodiode array detector (DAD-3000RS). The separation was performed using Acclaim® C<sub>18</sub> (5 μm) Dionex column (4.6 x 250 mm) at 30°C with a flow rate of 1 mL/min and an injection volume of 20 μL. The mobile phase consisted of acetonitrile (solvent A), acetic acid solution pH 3.0 (solvent B), and methanol (solvent C) with the

gradient elution program of 5% A/95% B (0-10 mins), 10% A/90% B (11-15 mins), 15% A/70% B/15% C (16-25 mins), 20% A/60% B/20% C (26-30 mins), 30% A/40% B/30% C (31-35 mins), 40% A/50% B/10% C (36-40 mins), and 5% A/95% B (41-45 mins). The UV detector was set to 280 nm for 25.0 mins, changed to 320 nm for 32.0 mins, again change to 280 nm for 34 mins and finally to 380 nm for 35 mins and held for the rest of the analysis period while the diode array detector was set at an acquisition range from 200 nm to 700 nm. For the preparation of calibration curve, a standard stock solution was prepared in methanol containing gallic acid, vanillic acid, myricetin, rosmarinic acid (8 µg/mL each); caffeic acid, syringic acid, vanillin, *trans*-ferulic acid, kaempferol (6 µg/mL each); (+)-catechin hydrate, (-)-epicatechin (10 µg/mL each); pyrogallol, ellagic acid (48 µg/mL each); *p*-coumaric acid (3 µg/mL); rutin hydrate (12 µg/mL) and quercetin hydrate (4 µg/mL). A solution of the ethanol extracts was prepared in methanol having the concentration of 10 mg/mL. Prior to HPLC analysis, all the solutions (mixed standards, sample, and spiked solutions) were filtered through 0.20 µm syringe filter (Sartorius, Germany) and then degassed in an ultrasonic bath (Hwashin, Korea) for 15 mins. Data acquisition, peak integration, and calibrations were calculated with Dionex Chromeleon software (Version 6.80 RS 10).

### 3. Results and discussion

#### 3.1 Total antioxidant capacity

Phosphomolybdate assay is an easy and well-known technique based on the reduction of Molybdenum (VI) to Molybdenum (V) by forming phosphate-Molybdenum (V) complex which is green in color with maximal absorption at a wavelength of 695 nm. Total antioxidant capacity of *C. olitorius* and *C. capsularis* is illustrated in Table 1. Results showed that *C. capsularis* leaves contained high total antioxidant capacity (214.32±1.95 mg of ascorbic acid/g of dry extract) then *C. olitorius* (165.66±1.30 mg of ascorbic acid/g of dry extract). Total antioxidant capacity is an analyte frequently used to assess the antioxidant status of biological samples and can evaluate the antioxidant response against the free radicals produced in a given disease (Rubio *et al.*, 2016).

Table 1. Total antioxidant capacity of 80% ethanol extract of Jute leaves (mg of ascorbic acid/g of dry extract)

Jute Leaves	Total antioxidant capacity
<i>C. olitorius</i>	165.66±1.30
<i>C. capsularis</i>	214.32±1.95

#### 3.2 Identification and quantification of polyphenols in *Corchorus olitorius* and *Corchorus capsularis* leaves

Identification and quantification of individual

polyphenolic compounds in the 80% ethanolic extracts of *C. olitorius* and *C. capsularis* were analysed by HPLC. The chromatographic separations of polyphenols in standard and 80% ethanolic extracts of *C. olitorius* and *C. capsularis* are shown in Figures 1, 2 and 3 respectively. The content of each polyphenolic compound found in the 80% ethanolic extracts of *C. olitorius* and *C. capsularis* was calculated from the corresponding calibration curve and presented as the mean of five determinations as shown in Tables 2 and 3.

The analysis of the results of HPLC-DAD allowed the detection of six and eight polyphenolic compounds from *C. olitorius* and *C. capsularis* leaves respectively. The experimental results indicated that 80% ethanolic extract of *C. olitorius* leaves contained an especially high concentration of rutin hydrate, ellagic acid, and quercetin hydrate (152.17±0.51, 143.27±0.58, and 292.83±0.73 mg/100 g of dry extract, respectively) than that of *C. capsularis* (32.16±0.08, 53.65±0.11, and 46.17±0.09 mg/100 g of dry extract, respectively). It was also shown that caffeic acid and vanillin were detected both in the 80% ethanol extract of *C. olitorius* and *C. capsularis* but the concentration of caffeic acid was at the moderate amount (51.06±0.11 and 55.93±0.13 mg/100 g of dry extract) and vanillin at a lower amount (5.18±0.04 and 1.04±0.01 mg/100 g of dry extract) shown in Tables 2 and 3. It was also found that vanillic acid, *trans*-ferulic acid and rosmarinic acid (13.28±0.05, 58.02±0.18 and 3.54±0.02 mg/100 g of dry extract, respectively) were detected only in the 80% ethanol extract of *C. capsularis* leaves and on the other side kaempferol (13.32±0.07 mg/100 g of dry extract) was detected only in the 80% ethanol extract of *C. olitorius* at a lower concentration shown in Tables 2 and 3. The major identified polyphenolic compounds of *C. olitorius* were rutin hydrate, ellagic acid and quercetin hydrate. These compounds display interesting biological properties, such as antioxidant as well as anti-inflammatory and anticancer activities (Selloum *et al.*, 2003; Vattem and Shetty, 2005; Anand David *et al.*, 2016; Ganeshpurkar and Saluja, 2017). The previous study reported the presence of ethanol, ethanol: water (50:50) and water extract of *C. olitorius* leaves contain caffeic acid (229.56, 146.02 and 306.43 mg/kg), quercetin (52.01, 35.26 and 3.13 mg/kg) and kaempferol (18.28, 29.39 and 16.24 mg/kg) etc (Ben Yakouba *et al.*, 2018). Which is lower than our present study. In *C. capsularis* leaves, the most abundant polyphenolic compounds were caffeic acid, *trans*-ferulic acid, rutin hydrate, ellagic acid and quercetin hydrate. These compounds have also a noticeable Pharmacological propriety such as antioxidant, anti-inflammatory and anticancer activities (Selloum *et al.*, 2003; Vattem and Shetty, 2005; Kumar and Pruthi, 2014; Anand David *et al.*, 2016;

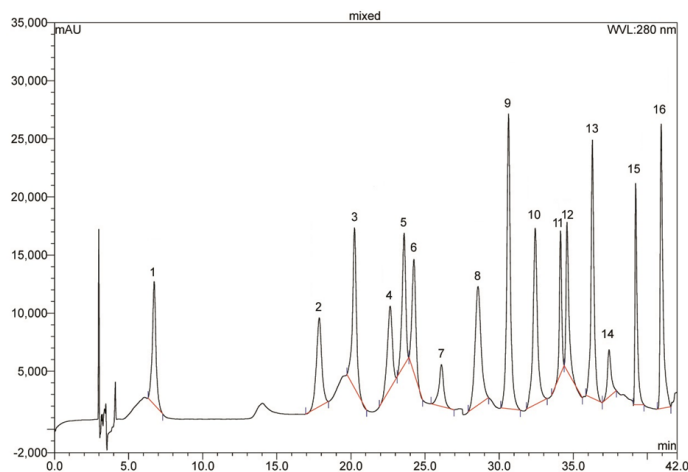


Figure 1. HPLC chromatogram of a standard mixture of polyphenolic compounds. Peaks: 1, gallic acid; 2, pyrogallol; 3, (+)-catechin hydrate; 4, vanillic acid; 5, caffeic acid; 6, syringic acid; 7, (-)-epicatechin; 8, vanillin; 9, *p*-coumaric acid; 10, *trans*-ferulic acid; 11, ellagic acid; 12, rutin hydrate; 13, rosmarinic acid; 14, myricetin; 15, quercetin hydrate; 16, kaempferol

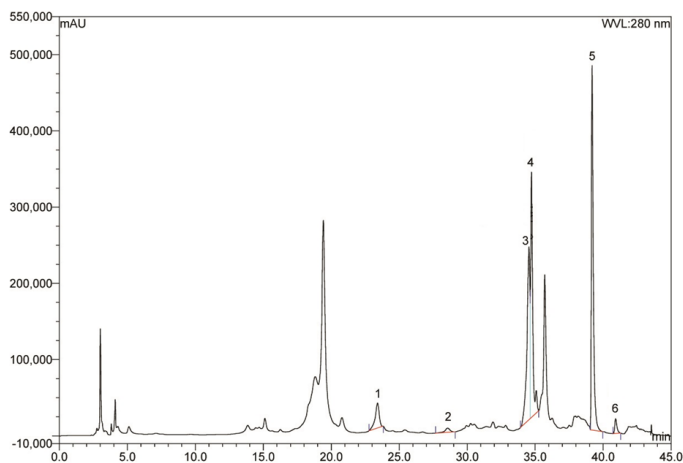


Figure 2. HPLC chromatogram of 80% ethanolic extract of *C. olitorius* leaves. Peaks: 1, caffeic acid (CA); 2, vanillin (VL); 3, rutin hydrate (RH); 4, ellagic acid (EA); 5, quercetin hydrate (QU); 6, kaempferol (KF)

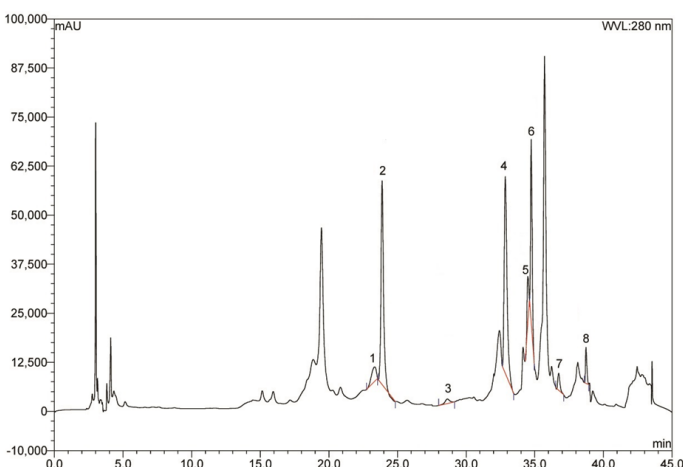


Figure 3. HPLC chromatogram of 80% ethanol extract of *C. capsularis* leaves. Peaks: 1, vanillic acid (VA); 2, caffeic acid (CA); 3, vanillin (VL); 4, *trans*-ferulic acid (FA); 5, rutin hydrate (RH); 6, ellagic acid (EA); 7, rosmarinic acid (RA); 8, quercetin hydrate (QU)

Ganeshpurkar and Saluja, 2017; Espíndola *et al.*, 2019). In this context, Mosihuzzaman *et al.* (1986) showed that *p*-coumaric, caffeic, vanillic, ferulic acid and *p*-hydroxybenzoic acids were present in the 80% aqueous ethanol extract of *C. capsularis* in unretted bark and stem of jute. The antioxidant is generally used to evaluate the total antioxidant power of single compounds and complex mixtures of different plants (Huang *et al.*, 2008). Antioxidant activity depends on the present of polyphenolic compounds (Materska and Perucka, 2005). Therefore, significant results of total antioxidant capacity of both extracts may be due present of different polyphenolic compounds.

Table 2. Contents of polyphenolic compounds in 80% ethanol extract of *C. olitorius* leaves (n=5)

Polyphenolic	Content (mg/100 g of dry extract)	% RSD
CA	51.06	0.11
VL	5.18	0.04
RH	152.17	0.51
EA	143.27	0.58
QU	292.83	0.73
KF	13.32	0.07

RSD: Relative Standard Deviation

Table 3. Contents of polyphenolic compounds in 80% ethanol extract of *C. capsularis* leaves (n=5).

Polyphenolic	Content (mg/100 g of dry extract)	% RSD
VA	13.28	0.05
CA	55.93	0.13
VL	1.04	0.01
FA	58.02	0.18
RH	32.16	0.08
EA	53.65	0.11
RA	3.54	0.02
QU	46.17	0.09

RSD: Relative Standard Deviation

#### 4. Conclusion

The results of the present study indicated that, *C. olitorius* and *C. capsularis* leaves exhibit a significant amount of total antioxidant capacity and polyphenolic compounds.

#### Conflict of interest

The authors declare no conflict of interest.

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